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(54) Title: BIOAVAILABLE COMPOSITION OF NATURAL AND SYNTHETIC HCA

(57) Abstract: The invention relates to a composition comprising hydroxycitric acid (HCA) in combination with either one or both of garcinol and anthocyanin, and its use as a weight-loss therapy in animal subjects, preferably humans. The therapeutic effects for the composition observed in murine and human studies include a reduction in total body weight and body mass index, a reduction in body fat, an increase in lean body mass and content of body water, and a reduction in perceived appetite level. Another composition for use in weight-loss therapy is also described relating to forskolin in combination with either one or both of garcinol and anthocyanin. The anti-oxidant properties of garcinol are described as being enhanced in the presence of HCA and anthocyanin, and the combination of HCA, garcinol and anthocyanin is also shown to exert greater citrate lyase inhibiting properties than either compound alone. Methods of obtaining HCA, garcinol or anthocyanin, or a composition containing all three compounds, are described.

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5 Bioavailable Composition of Natural and Synthetic HCA

This application claims priority under 35 U.S.C. §1.119(e) to provisional application serial no. 60/225,821, filed on August 17, 2000.

10 Background of the invention

Hydroxycitric acid is an alpha-hydroxy tribasic acid (1,2-dihydroxypropane-1,2,3-tricarboxylic acid) with two asymmetric centers, hence the formation of two pairs of diastereoisomers or four different isomers: (-)hydroxycitric acid (I), (+)hydroxycitric acid (II), (-)allo-hydroxycitric acid (III), and (+)allo-hydroxycitric acid (IV). (1-2) The (-)hydroxycitric acid (HCA) isomer is found in the rind of *Garcinia cambogia* fruit (fam. Clusiaceae). (1-2) This isomer has been shown to be a potent linear competitive inhibitor of ATP citrate lyase enzyme, *in vitro*, demonstrating a much greater affinity for the purified enzyme than its natural substrate citrate as well as the other stereoisomers of hydroxycitric acid. (1-2) The biological importance of ATP citrate lyase is as a citrate cleavage enzyme which catalyzes the extramitochondrial cleavage of citrate to acetyl CoA and oxaloacetate, and facilitates the biosynthesis of fatty acids. The reversible inhibition of citrate lyase by (-) HCA may lead to the reduction of fatty acids synthesis and lipogenesis. These effects have been measured and demonstrated *in vivo* following the oral, intravenous or intraperitoneal administration of (-)hydroxycitrate to experimental animals. (3) When (-) HCA was given orally before the feeding period, the animals fed (-) HCA consumed less food and their hepatic synthesis of fatty acids and cholesterol was significantly diminished as compared to the untreated controls. (3-4) The observed decrease in food intake may be only one of the factors responsible for the (-) HCA promoted weight loss, because experimentation with rats fed (-) HCA showed weight loss with no decrease in cumulative food intake. (5) It seems that the potential mechanism of weight loss with (-) HCA may include an energy expenditure component, the nature of which remains undetermined. (5) This mechanism of energy expenditure, decreased lipogenesis, and the reduction in food intake in (-) HCA-treated animals may result in loss of weight and total body fat content. (6)

Although the potential of (-)HCA as a weight lowering compound has been recognized since the 1970's, only few clinical studies have been conducted with this compound. (7-12). These few studies examining HCA-mediated prevention of excess body fat, resulted in contradictory results, most likely due to HCA being poorly bioavailable in the cytosol of a target cell. In one clinical study of HCA, a controversial high fiber diet was used. The use of a high-fiber diet in combination with HCA may reduce gastrointestinal

5 absorption of HCA, since high-fiber diets are known to reduce absorption of many nutrients and micronutrients. This issue becomes critical with HCA because its reported efficacy in inhibiting the intracellular enzyme, adenosine triphosphate (ATP)-citrate-lyase, depends entirely on the presence of HCA inside the target cell.

10 In their U.S. Patent, the Inventors addressed an important issue regarding the bioavailability of the HCA compounds. The 5,783,603 patent described a manufacturing process leading to a unique structure for a potassium salt of HCA, which facilitated its transport across biological membranes, effectively delivering more HCA into the cytosol for the competitive inhibition of ATP citrate lyase. Although the '603 patent related to an HCA compound having considerably improved bioavailability, its bioavailability was still
15 relatively inefficient. For example, an in vitro study done on hepatic cells, indicates that 5 mM of extracellular potassium HCA could inhibit ATP citrate lyase. However, only 0.5 mM of potassium HCA is actually needed in the cytosol to effectively inhibit ATP citrate lyase. Therefore, a 10-fold excess amount of potassium HCA is needed outside of the target cell in order to achieve a concentration of 1/10 that amount in the cytosol. This finding of relatively
20 poor bioavailability of HCA, was confirmed in pre-clinical experiments (14), and points out the need to further improve the bioavailability and efficacy of HCA.

Garcinol, like HCA, is isolated from *Garcinia* sp. fruit rind, and it exhibits anti-oxidant and chemoprotective properties (15). In one experiment, rats fed a garcinol diet (0.01% and 0.05%) showed a significantly reduced development of azoxymethane (AOM)-
25 induced colonic aberrant crypt foci (ACF) as compared to control animals. Feeding of garcinol significantly elevated liver glutathione S-transferase, quinone reductase activities, suppressed O₂- and NO generation and expression of iNOS and COX-2 proteins. These findings suggest a possible chemopreventive mechanism of garcinol.

Garcinol and isogarcinol were evaluated for their antibacterial activity against
30 methicillin-resistant *Staphylococcus aureus* (16). These compounds showed a minimum inhibitory concentration at 3.1-12.5 micrograms/ml, or nearly equal to that of the antibiotic, vancomycin.

In 1981, Krishnamurthy et al. (17) reported the isolation of garcinol, and its colorless isomer, isogarcinol, from *Garcinia indica*. Their structures were proposed on the basis of
35 chemical and spectral data. Garcinol, C₃₈H₅₀O₆, m.p. 122(o), crystallized out from the hexane extract of the fruit rind of *G. indica* as yellow needles (1.5 percent). The UV spectrum of garcinol suggested that the 1,3-diketone system is conjugated to the 3,4-dihydroxybenzoyl moiety. The IR spectrum of the trimethyl ether showed the presence of a saturated carbonyl group (1727 cm⁻¹) and two α,β -unsaturated carbonyl groups (1668 and 1642 cm⁻¹).

5 Isogarcinol was isolated by column chromatography of the extract. Its identity was established by mixed m.p. and by comparison of UV, IR, and PMR spectra. The IR spectrum of isogarcinol indicated the presence of saturated carbonyl group (1715 cm⁻¹), an aroyl group (1670 cm⁻¹) and an α,β -unsaturated carbonyl group (1635 cm⁻¹).

10 Rao et al. (18) reported the isolation of cambogin (33H55O6), m.p. 242-244, from the latex of *Garcinia cambogia* tree. The structure was confirmed by UV, IR and NMR studies. UV: 231-234, 275-278 and 305-313; IR (KBr): 1720 (saturated carbonyl), 1680 and 1642 cm⁻¹ (unsaturated carbonyl and double bond). Besides cambogin they also reported the isolation of camboginol and related its structure to cambogin.

15 In 1982, N. Krishnamurthy et al. (19) isolated anthocyanin pigments from the fresh red ripe fruits of Kokam (*Garcinia indica*). The rind portion was separated from the rest of the fruit and was macerated in a blender using methanol containing one percent HCl for three times. The extracts were combined, filtered and concentrated in vacuo at 30°C. Paper chromatography of the Kokam pigment extract showed two anthocyanin bands. The slower moving band was designated as B1 and the other B2. The total anthocyanin concentration 20 was estimated to be 2.4 percent on a dry weight basis; the ratio of B1 to B2 is 1:4.

Anthocyanin B1 was identified as cyanidine-three-glucoside by chemical and spectroscopic studies. This compound on hydrolysis gave cyaniding and glucose. The UV spectral maximum (527 nm) of the glycosides shifted to 567 nm with aluminium chloride indicating that 3'- and 4'-hydroxyl groups of the cyaniding are free. The structure was 25 confirmed by direct comparison with a sample of cyaniding-three-glucoside obtained from mulberry.

Anthocyanin B2 was identified as cyaniding-three-sambubioside. This anthocyanin on complete hydrolysis gave cyaniding, glucose and xylose. The spectral data suggested that B2 is a three-substituted glycoside of cyaniding. Hydrogen peroxide hydrolysis removed the 30 disaccharide from the pigment which on further acid hydrolysis gave glucose and xylose. The structure was confirmed by direct comparison with a sample of cyaniding-3-sambubioside isolated from Roselle.

The present invention is based on the unexpected finding that combining HCA with natural compounds obtained from *Garcinia* sp. plant including garcinol (polyisoprenylated 35 benzophenone) and/or anthocyanin compounds, results in not only an enhancement of the biological activity of HCA but also that of garcinol and/or anthocyanin.

5 Brief Description of the Figures

- Figure 1: Trendlines of the percentage of subjects who experienced weight loss.
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20
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25
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30 intervals.
- Figure 19: Diastolic blood pressure in groups C and NC in consecutive study time intervals.

Detailed Description of the Invention

- 35 The invention relates to a composition comprising hydroxycitric acid (HCA) in combination with either one or both of garcinol and anthocyanin and having the effect of increasing bioavailability of HCA in the cytosolic compartment of a cell.

Another object of the present invention, involves the use of the inventive composition comprising HCA in combination with either one or both of garcinol and anthocyanin, as a

5 weight-loss therapy in animal subjects, preferably humans. The therapeutic effects for the composition observed in murine and human studies include a reduction in total body weight and body mass index, a reduction in body fat, an increase in lean body mass and content of body water, and a reduction in perceived appetite level. The combination of the compounds is also shown to exert greater citrate lyase inhibiting properties than either compound alone.

10 Garcinol, alone, is also shown to have chemopreventive properties in an animal tumor model.

Biological activity includes but is not limited to activity for appetite suppression, inhibition of cytoplasmic citrate lyase, enhanced fat catabolism, and increased anti-oxidant activity.

15 Hydroxycitric acid (HCA) is an alpha-hydroxy tribasic acid (1,2-dihydroxy propane - 1, 2, 3-tricarboxylic acid) comprising (-)hydroxycitric acid (I), (+) hydroxycitric acid (II), (-) allo-hydroxycitric acid (III), and (+) allo-hydroxycitric acid (IV).

Garcinol is a polyisoprenylated benzophenone (PPB) derived from *Garcinia* sp. Garcinol is a well-known anti-oxidant (i.e., emulsified garcinol suppressed superoxide anion comparably to DL alpha-tocopherol), anti-carcinogen and also has anti-microbial properties.

20 Anthocyanins are one class of flavenoid compounds, which are widely distributed plant polyphenols. There is considerable evidence that dietary anthocyanin pigments have preventative and therapeutic roles in a number of human diseases. The chemical basis for these desirable properties of anthocyanins is believed to be related to their antioxidant capacity- their ability to scavenge and trap free radicals that damage biomolecules.

The combination of garcinol and HCA enhances bioavailability of HCA in the cytosol and this same combination also enhances the antioxidant properties of garcinol. This mutual enhancement of the biological properties for the compounds of the invention, is referred to as "a biological effect amplification" or "Amplibio".

30 Amplibio refers to a mechanism wherein either one of the compounds serves to facilitate the biological effect of other compounds and as a result, both biological activities are enhanced.

In many biological systems, interaction of two or more components may result in no interaction, antagonism, synergism or a distinct form of synergism called amplibio. Not all compounds that exert synergism exert amplibio action. For example, there is an advantage in combining alpha lipoic acid with vitamins E and/or C, because lipoic acid recycles both vitamins, making them more bioavailable to the body. However, both vitamins do not enhance or otherwise improve the action of the lipoic acid. On the other hand, the results obtained by combining polyunsaturated fatty acids with fat soluble vitamins exemplifies an

5 Amphibio effect: Vitamin E preserves fatty acids by preventing oxidation, while fatty acids enhance absorption of vitamin E. Another example of an Amphibio effect is observed when supplemental nutrients are combined with compounds endogenously produced by the living body: curcuminoids are known to enhance glutathione levels in the body, which occurs through regulating the rate of glutathione use in glutathione-free radical reaction. Elevated
10 glutathione, in turn, increases the availability of curcuminoids in the body.

The Amphibio action of the present invention may involve the following mechanisms:

- the anti-oxidant action of garcinol/anthocyanin (enhanced by HCA) neutralizing negative charges surrounding the cell to facilitate transport and uptake of HCA into the cell;
- 15 • garcinol/anthocyanin affecting fluidity of cell membranes to facilitate transport and uptake of HCA into the cell;
- garcinol/anthocyanin synergizing with HCA to inhibit citrate lyase, thus lowering the threshold for such inhibition with the HCA.

Therefore, enhancement of HCA activity is shown to be effected by its interaction
20 with garcinol/anthocyanin, likewise, HCA is observed to enhance the biological potential of garcinol and anthocyanin. The qualitative and quantitative interaction among the three compounds of the invention have not been described previously.

Methods for extracting and purifying HCA, garcinol and anthocyanin compounds are well known in the art.

25 The present invention also includes a method of preparing a composition comprising PPB, anthocyanin and HCA extracted from fruits of *Garcinia* sp., the method comprising:

- (a) extracting the fresh fruits of *Garcinia* with 8 to 15 parts of C1 to C6 alcohol (e.g. methanol, ethanol, isopropyl alcohol) by refluxing and circulating in a
30 closed reactor until extraction is completed;
- (b) filtering the extracted material from the spent material;
- (c) concentrating the extracted material to $\frac{1}{4}$ - $\frac{1}{6}$ its volume under vacuum at 540 to 600 mm/Hg;
- (d) chilling the concentrate for approx. 20 to 30 hours at -5 to 5°C and filtering
35 the precipitated material in a Nutsche filter;
- (e) obtaining a solid residue (yield 2.5 to 4%) comprising crude Garcinol (Assay 16-20%).

- 5 (f) dissolving crude Garcinol extract in a hydrocarbon solvent such as petroleum ether, hexane or toluene (3 to 8 parts), and extracting/partitioning with a high polar, non-miscible solvent such as acetonitrile (2 to 4 parts, 3 to 5 times);
- (g) separating and concentrating the polar solvent layer to $\frac{1}{4}$ - $\frac{1}{6}$ its volume, and chilling at -5 to 8°C for 12 to 16 hours;
- 10 (h) filtering and washing the residue with the polar solvent to obtain yellow Garcinol (yield=0.8 to 1.5%, Assay 55 to 70%);
- (i) adding to the extracted material of step c), molar equivalent quantities of alkali (sodium hydroxide or potassium hydroxide) calculated on the basis of the content of HCA (about 20 to 25%);
- 15 (j) heating the mixture under stirring at 80 to 95°C for 2.5 to 5 hours to obtain yellow PPB (Garcinol);
- (k) filtering the precipitated material (HCA salt of sodium or potassium) using a Nutsche filter and washing with cold alcohol;
- (l) dissolving the precipitated material in 3 to 5 parts of water;
- 20 (m) adding to this aqueous solution, molar equivalent quantity of calcium chloride and heating with stirring at 80 to 95°C for 4 to 6 hours;
- (n) filtering and drying the precipitated material at 50 to 75°C under vacuum to obtain white HCA calcium (yield=25 to 35%);
- (o) blending the yellow PPB (Garcinol) powder obtained in step (j) and the HCA calcium obtained in step (n) at a ratio of 1:10 to yield a composition containing HCA calcium at a minimum of 55% and PPB (Garcinol) at a minimum of 5%; and
- 25 (p) optionally blending HCA calcium or any other salt of HCA, with PPB and proantho-cyanidins in effective proportions at a minimum of 0.5% of the proanthocyanidins.
- 30

The PPB composition of the invention can also be obtained as follows:

- 35 (a) extracting Garcinia spent fruit from suspension with toluene and 5% methanol to obtain a paste having 20% PPB's (Garcinol - 15%; Cambogin - 5%);
- (b) upgrading the paste to obtain a composition comprising 50% Garcinol and approx. 10-15% Cambogin which comes in the mother liquor, and 50% Garcinol is upgraded further by one more crystallization to yield 90% Garcinol plus 35% cambogin;

- 5 (c) heating a 90% Garcinol composition to 100°C for conversion to Cambogin of about 80% proof, the remainder being Garcinol;
- (d) mixing the product of step (c) in equal proportion with part of a 90% Garcinol composition to obtain a 85% - 90% PPB composition with 42 – 45% proof;
- 10 (e) obtaining a final composition with a 90% PPB in almost equal proportion to Cambogin and Garcinol, the remainder being cyclised products called poly isoprenylated xanthenes.

The term "bioavailability" is defined as any means to enhance the effective mechanism of a weight loss compound. By analogy, the bioavailability of calcium in the
15 body is ultimately measured by increased bone mass, which means that any mechanism serving the purpose of incorporating the supplemental calcium in the bone will increase bioavailability of calcium.

The term "effective amount" means a sufficient amount of compound, e.g. corresponding to HCA, garcinol or anthocyanin delivered to produce an adequate level of
20 cytosolic citrate lyase.

A primary object of the invention is to provide a method of therapy where the bioavailability of HCA is dramatically increased to obtain therapeutically effective amounts of HCA in the cytosol, thereby treating the subject in need of reducing or inhibiting citrate
25 lyase. This enhanced activity for HCA is achieved by co-administration with compounds including but not limited to garcinol and anthocyanin.

Another object of the invention is to provide a method of weight-loss therapy where the effects of forskolin (USPN 5,804,596) are dramatically increased by the presence of garcinol and, optionally, anthocyanin. The amount of forskolin is the same as taught in the '596 patent, and the amounts of garcinol and anthocyanin are the same as taught herein for
30 use with HCA.

More specifically, a weight loss and appetite suppressing effective amount of HCA and garcinol is 500 mg tid and 25 mg respectively, more preferably the dose of garcinol can range from 0.01% to 10% of the HCA or other weight loss compounds like diterpene
35 forskolin (USPN 5,804,596). A weight loss effective amount of HCA and anthocyanin is 500 mg tid and 10 mg respectively, more preferably the dose of anthocyanin can range from 0.01% to 10% of the HCA or other weight loss compounds like diterpene forskolin (USPN 5,804,596). A weight loss and appetite suppressing effective amount of HCA, garcinol and anthocyanin is 500 mg, 25 mg and 10 mg respectively, more preferably the dose of garcinol

5 and anthocyanin can range from 0.01% to 10% of the HCA or other weight loss compounds like diterpene forskolin (USPN 5,804,596).

A citrate lyase inhibiting effective amount of HCA and garcinol is 500 mg tid and 25 mg respectively, more preferably the dose of garcinol can range from 0.01% to 10% of the HCA or other weight loss compounds like diterpene forskolin (USPN 5,804,596). A citrate
10 lyase inhibiting effective amount of HCA, and anthocyanin is 500 mg tid and 10 mg respectively, more preferably the dose of anthocyanin can range from 0.01% to 10% of the HCA or other weight loss compounds like diterpene forskolin (USPN 5,804,596). A citrate lyase inhibiting effective amount of HCA, garcinol and anthocyanin 500 mg, 25 mg and 10 mg respectively, more preferably the dose of garcinol and anthocyanin can range from 0.01%
15 to 10% of the HCA or other weight loss compounds like diterpene forskolin (USPN 5,804,596).

A fat catabolizing and lean body mass enhancing effective amount of HCA and garcinol is 500 mg tid and 25 mg respectively, more preferably the dose of garcinol can range from 0.01% to 10% of the HCA or other weight loss compounds like diterpene forskolin
20 (USPN 5,804,596). A fat catabolizing and lean body mass enhancing effective amount of HCA and garcinol is 500 mg tid and 25 mg respectively, more preferably the dose of garcinol can range from 0.01% to 10% of the HCA or other weight loss compounds like diterpene forskolin (USPN 5,804,596). A fat catabolizing and lean body mass effective amount of HCA, garcinol and anthocyanin is 500 mg, 25 mg and 10 mg respectively, more preferably
25 the dose of garcinol and anthocyanin can range from 0.01% to 10% of the HCA or other weight loss compounds like diterpene forskolin (USPN 5,804,596).

The therapeutic weight-loss effects of HCA and garcinol, in combination, and the role of garcinol as chemopreventive agent are described by way of the following non-limiting examples.

30

Example 1

Administration of garcinol and HCA to two strains of mice, SKH-1 and CF-1 respectively, resulted in significantly less total body weight and abdominal fat gain, as compared to control, chow-receiving animals and the groups of animals receiving either garcinol or HCA
35 alone. In addition, dietary administration of garcinol caused significant reduction in aberrant colonic crypt formation (AC is considered a pre-malignant condition) in CF-1 mice as compared to the animals fed control diet or diet containing HCA or garcinol alone. Interestingly, the weight-gain preventive effect of garcinol and HCA combination was

- 5 accomplished despite that the garcinol plus HCA animals had higher food and water consumption than the control, garcinol and HCA groups.

10 Table 1. Effect of oral administration of garcinol (GAR), hydroxycitric acid (HCA) and combination of garcinol and hydroxycitric acid on body weight, food and fluid consumption in SKH-1 mice

Group		5 weeks	7 weeks	10 weeks
Body weight (gm; Mean±SE)				
15	1. Control	32.3±0.67	34.5±0.99	37.0±1.49
	2. 0.05% GAR	32.8±0.37	33.9±0.14	36.4±0.55
	3. 1% HCA	32.5±0.42	34.5±0.59	36.8±0.33
	4. (2)+(3)	31.2±0.20	33.5±0.58	34.9±0.88
Food consumption (gm/mouse/day; Mean±SE)				
	1. Control	5.25±0.33	5.09±0.28	5.16±0.25
	2. 0.05% GAR	4.98±0.17	5.09±0.46	5.26±0.17
	3. 1% HCA	5.74±0.13	6.49±0.07	6.93±0.21
25	4. (2)+(3)	6.55±0.20	8.03±1.45	9.31±1.12
Water consumption (ml/mouse/day; Mean±SE)				
	1. Control	3.75±0.04	3.42±0.34	3.80±0.22
	2. 0.05% GAR	3.66±0.07	3.63±0.05	3.73±0.17
30	3. 1% HCA	3.72±0.08	3.74.5±0.04	3.84±0.09
	4. (2)+(3)	3.68±0.08	3.86±0.08	3.94±0.08

35 Table 2. Effect of 10 week oral administration of garcinol (GAR) and hydroxycitric acid (HCA) on azoxymethane (AOM)-induced formation of aberrant colonic crypts (AC) and accumulation of fat in abdomen in CF-1 mice

Group	Body weight (gm)	AC per colon	parametrial fat (gm)	retroperitoneal fat (gm)
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1. Control	38.8±1.52	11.4	1.33±0.19	0.95±0.11
2. 0.05% GAR	37.3±1.07	7.8 (31.6%)	1.21±0.18	0.85±0.01
3. 1% HCA	37.9±0.66	7.9 (30.7%)	1.24±0.06	0.79±0.04
4. (2)+(3)	36.1±0.48	8.5 (25.4%)	0.95±0.10	0.70±0.13

10

These data suggest a broad biological mechanism for garcinol as an adjuvant to HCA involving regulation of body weight, body composition and body metabolic rate.

Example 2

15 The role of garcinol as a chemopreventive agent has been confirmed in an experiment in which CF-1 mice were treated topically with the inflammation and tumor promoting agent, TPA.

Table 3. Effects of garcinol (GAR) on TPA-induced ear edema*

20 Treatment	Number of mice	Weight per punch (gm)	Percent Inhibition (%)
1. TPA (1nmol)	5	15.5±0.82	-
2. TPA + GAR (0.1 umol)	4	12.4±0.81	38.5
25 3. TPA + GAR (0.5 umol)	4	9.25±0.66	77.5

* Female CF-1 mice were treated topically with TPA applied to ear's skin with or without garcinol. Five hours later, the mice were killed and ear punches were weighted. Data are expressed as the mean±SE.

30 These data suggest a biological mechanism for garcinol as a chemopreventative agent.

Example 3

Clinical studies on weight-loss potential of garcinol and HCA

35 The combination consisting of 500 mg of calcium salt of HCA and 25 mg of garcinol (NC) was evaluated in a double-blind, 12 week clinical study against the formula containing 500 mg of calcium salt HCA (C). The study was performed on 46 overweight female volunteers (BMI greater than 25). Participants were instructed to take one capsule of either active-

5 containing or placebo formula three times a day, half an hour before a meal. Each participant was asked to maintain her previous daily physical exercise and eating habits. In addition, physical activity was monitored based on a questionnaire before and during the trial. The participants were evaluated at the baseline, weeks 2,4,6,8,10 and 12. The following clinical parameters were evaluated at each visit: total body weight, body composition by the
10 bioelectric impedance method, self-assessed appetite and energy levels, pulse rate and blood pressure. During the 12 week trial, the mean values in group NC for body weight and fat content significantly decreased, whereas lean body mass and total body water significantly increased compared to the baseline values and C group values. The appetite levels were significantly less in the NC group than the C group, whereas energy levels were equally
15 increased in both study groups as compared to the baseline. No subjective or objective adverse effects were reported in the course of this study. The pulse rate, systolic and diastolic blood pressure were maintained at the same level throughout the study.

NC is statistically more effective than C in reducing total body weight and body mass index; reducing body fat; increasing lean body mass and content of body water; and reducing
20 levels of appetite perception. The energy levels of subjects were not enhanced by regimens in both groups, and NC and C did not produce subjective or objective side effects.

Industrial Applications

The combined data from animal and clinical studies indicate that the invention is
25 more effective than HCA alone weight-loss therapy, and that in addition to fat loss, the inventive composition invention also improves lean body mass leading to improved body composition. Maintaining or improving lean body mass while shedding extra pounds of adipose tissue has been advocated by the Centers for Disease Control. Lean body mass is important because it has been recently recognized as an independent, positive predictor of
30 one's cardiovascular health and overall health. The chemopreventive action of garcinol in the animal experimental model may also be intricately related to the role the compound has in improving body composition.

5 References

1. Sullivan AC, Singh M, Srere PA, Glusker JP. Reactivity and Inhibitor Potential of Hydroxycitrate Isomers with Citrate Synthase, Citrate Lyase, and ATP Citrate Lyase. *J Biol Chem.* 1977; 252(21): 7583-7590.
- 10 2. Majeed M, Rosen R, McCarthy M, Conte AA, Patil D, Butrym E. Citrin A Revolutionary Herbal Approach to Weight Management. New Editions Publishing. Burlingame, CA. 1994.
3. Sullivan AC, Hamilton JG, Miller ON, Wheatley. Inhibition of lipogenesis in rat liver by
15 (-)-hydroxycitrate. *Arch Biochem Biophys.* 1972; 150: 183-190.
4. Sullivan AC, Triscari J, Hamilton JG, Miller ON. Effect of (-)-Hydroxycitrate upon the Accumulation of Lipid in the Rat: I. Lipogenesis. *Lipids.* 1973; 9(2):121-128.
- 20 5. Vasselli JR, Shane E, Boozer CN, Heymsfield SB. Garcinia Cambogia Extract Inhibits Body Weight Gain Via Increased Energy Expenditure (EE) In rats. *FASEB J.* 1998;12(part1):A506.
6. Sullivan AC, Triscari J, Hamilton JG, Miller ON. Effect of (-)-Hydroxycitrate upon the
25 Accumulation of Lipid in the Rat: II. Appetite. *Lipids.* 1973;9(2):121-128.
7. Conte A.A. A Non-Prescription Alternative in Weight Reduction Therapy. *The Bariatrician.* 1993: 17-19.
- 30 8. Conte A.A. The effects of (-) - Hydroxycitrate And Chromium (GTF) On Obesity. *J Amer Coll Nutr.* 1994 (October);13 (5): 535 [Abstract 60].
9. Katts GR, Pullin D, Parker LK, Keith PL, Keith S. Reduction Of Body Fat As A Function Of Taking A Dietary Supplement Containing Garcinia Cambogia Extract, Chromium
35 Picolinate And L-Carnitine - A Double Blind Placebo Controlled Study. Abstract/Poster presented at a symposium on obesity organized by the Mexican Sociedad Medical del Sureste para el Estudio de la Obesidad, March 4, 1995, Merida, Yucatan, Mexico.

- 5 10. Badmaev V, Majeed M. Open Field, Physician Controlled, Clinical Evaluation Of Botanical Weight Loss Formula Citrin(r). Nutracon 1995: Nutraceuticals, Dietary Supplements And Functional Foods. Day One (Sponsored by Global Business Research LTD). Published in the symposium book.
- 10 11. Thom E. (-)Hydroxycitrate (HCA) In The Treatment Of Obesity. *Int J Obesity*. 20 (4): 75 [Abstract /Poster 08-193-WP1 at 7th European Congress on Obesity in Barcelona, Spain 14-17 May, 1996].
12. Heymsfield SB, Allison DB, Vasselli JR, Pietrobelli A, Greenfield D, Nunez C. *Garcinia cambogia* (hydroxycitric acid) as a potential antiobesity agent: a randomized controlled trial. *JAMA*. 1998;280:1596-1600.
- 15 13. Majeed M., Badmaev V., Rajendran R. Potassium Hydroxycitrate For The Suppression Of Appetite And Induction Of Weight Loss. US Patent 5, 783, 603.
- 20 July 21, 1998.
14. Puttaparthi K, Rogers T, Elshourbagy NA, Levi M, Melnick JZ. Renal ATP citrate lyase (ATP CL) protein localizes throughout the nephron and increases only in the proximal tubule with chronic metabolic acidosis (CMA). Abstract presented at Pediatric Academic Societies' 25 1999 Annual Meeting. May 2, 1999; San Francisco, Calif.
15. Tanaka, T. et al. Prevention of colonic aberrant crypt foci by dietary feeding of garcinol in male F3444 rats. *Carcinogenesis* 2000 Jun;21(6):1183-9.
- 30 16. Iinuma M et al. Antibacterial activity of some *Garcinia* benzophenone derivatives against methicillin-resistant *Staphylococcus aureus*. *Biol Pharm Bull* 1996 Feb;19(2):311-4.
17. Krishnamurthy, N. et al. *Tetrahedron Lett*. 1981; 793.
18. Rao Rama, AV. et al. *Chem Ind*. 1979; 92.
19. Krishnamurthy, N. et al. *J Food Sci and Tech*. 1982;97:19.

What is claimed:

1. A method for appetite suppression in a subject in need of such effect, comprising administering to said subject an appetite suppressing effective amount of hydroxycitric acid and garcinol.
2. A method for appetite suppression in a subject in need of such effect, comprising administering to said subject an appetite suppressing effective amount of hydroxycitric acid and anthocyanin.
3. A method for appetite suppression in a subject in need of such effect, comprising administering to said subject an appetite suppressing effective amount of hydroxycitric acid, garcinol and anthocyanin.
4. A method for inhibiting cytoplasmic citrate lyase in a subject in need of such effect, comprising administering to said patient a citrate lyase inhibiting effective amount of hydroxycitric acid and garcinol.
5. A method for inhibiting cytoplasmic citrate lyase in a subject in need of such effect, comprising administering to said subject a citrate lyase inhibiting effective amount of hydroxycitric acid and anthocyanin.
6. A method for inhibiting cytoplasmic citrate lyase in a subject in need of such effect, comprising administering to said subject a citrate lyase inhibiting effective amount of hydroxycitric acid, garcinol and anthocyanin.
7. A method for increasing fat catabolism and increasing lean body mass in a subject in need of such effect comprising administering to said subject a fat catabolizing effective amount of hydroxycitric acid and garcinol.
8. A method for increasing fat catabolism and increasing lean body mass in a subject in need of such effect comprising administering to said subject a fat catabolizing effective amount of hydroxycitric acid and anthocyanin.
9. A method for increasing fat catabolism and increasing lean body mass in a subject in need of such effect comprising administering to said subject a fat catabolizing effective amount of hydroxycitric acid, garcinol and anthocyanin.
10. A method for increasing anti-oxidant activity of garcinol in a subject in need of such effect, comprising administering to said subject an anti-oxidant effective amount of garcinol, hydroxycitric acid and anthocyanin.
11. A method for increasing anti-oxidant activity of anthocyanin in a subject in need of such effect comprising administering to said subject an anti-oxidant effective amount of anthocyanin, hydroxycitric acid and garcinol.
12. A process of manufacturing hydroxycitric acid from fruits of *Garcinia* sp. comprising extracting said HCA from the fruits with organic solvents.

13. A process of manufacturing garcinol from fruits of *Garcinia* sp. comprising extracting said garcinol from the fruits with organic solvents.
14. A process of manufacturing of anthocyanin from fruits of *Garcinia* sp. comprising extracting said anthocyanin from the fruits with organic solvents.
15. A process of manufacturing hydroxycitric acid (HCA), garcinol and anthocyanin from fruits of *Garcinia* sp. comprising extracting said HCA, garcinol and anthocyanin from the fruits with organic solvents.
16. A process of manufacturing hydroxycitric acid from fruits of *Garcinia* sp. by CO₂ supercritical extraction.
17. A process of manufacturing garcinol from fruits of *Garcinia* sp. by CO₂ supercritical extraction.
18. A process of manufacturing anthocyanin from fruits of *Garcinia* sp. by CO₂ supercritical extraction.
19. A process of manufacturing hydroxycitric acid, garcinol and anthocyanin from fruits of *Garcinia* sp. by CO₂ supercritical extraction.
20. A method for appetite suppression in a subject in need of such effect, comprising administering to said subject an appetite suppressing effective amount of forskolin and garcinol.
21. A method for appetite suppression in a subject in need of such effect, comprising administering to said subject an appetite suppressing effective amount of forskolin and anthocyanin.
22. A method for appetite suppression in a subject in need of such effect, comprising administering to said subject an appetite suppressing effective amount of forskolin, garcinol and anthocyanin.
23. A method for inhibiting cytoplasmic citrate lyase in a subject in need of such effect, comprising administering to said patient a citrate lyase inhibiting effective amount of forskolin and garcinol.
24. A method for inhibiting cytoplasmic citrate lyase in a subject in need of such effect, comprising administering to said subject a citrate lyase inhibiting effective amount of forskolin and anthocyanin.
25. A method for inhibiting cytoplasmic citrate lyase in a subject in need of such effect, comprising administering to said subject a citrate lyase inhibiting effective amount of forskolin, garcinol and anthocyanin.
26. A method for increasing fat catabolism in a subject in need of such effect comprising administering to said subject a fat catabolizing effective amount of forskolin and garcinol.
27. A method for increasing fat catabolism in a subject in need of such effect comprising administering to said subject a fat catabolizing effective amount of forskolin and anthocyanin.
28. A method for increasing fat catabolism in a subject in need of such effect comprising administering to said subject a fat catabolizing effective amount of forskolin, garcinol and anthocyanin.

Figure 1

Trendlines of the percentage of subjects who experienced weight loss

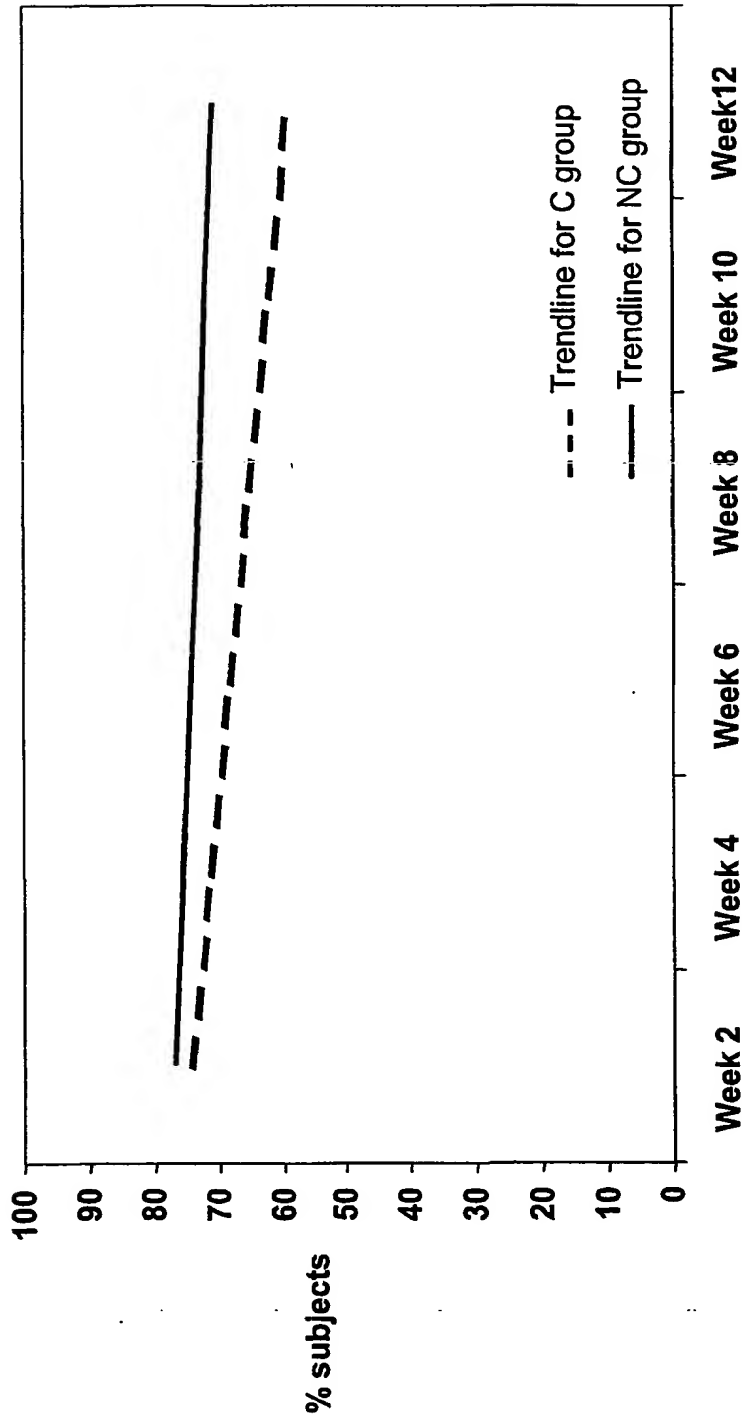
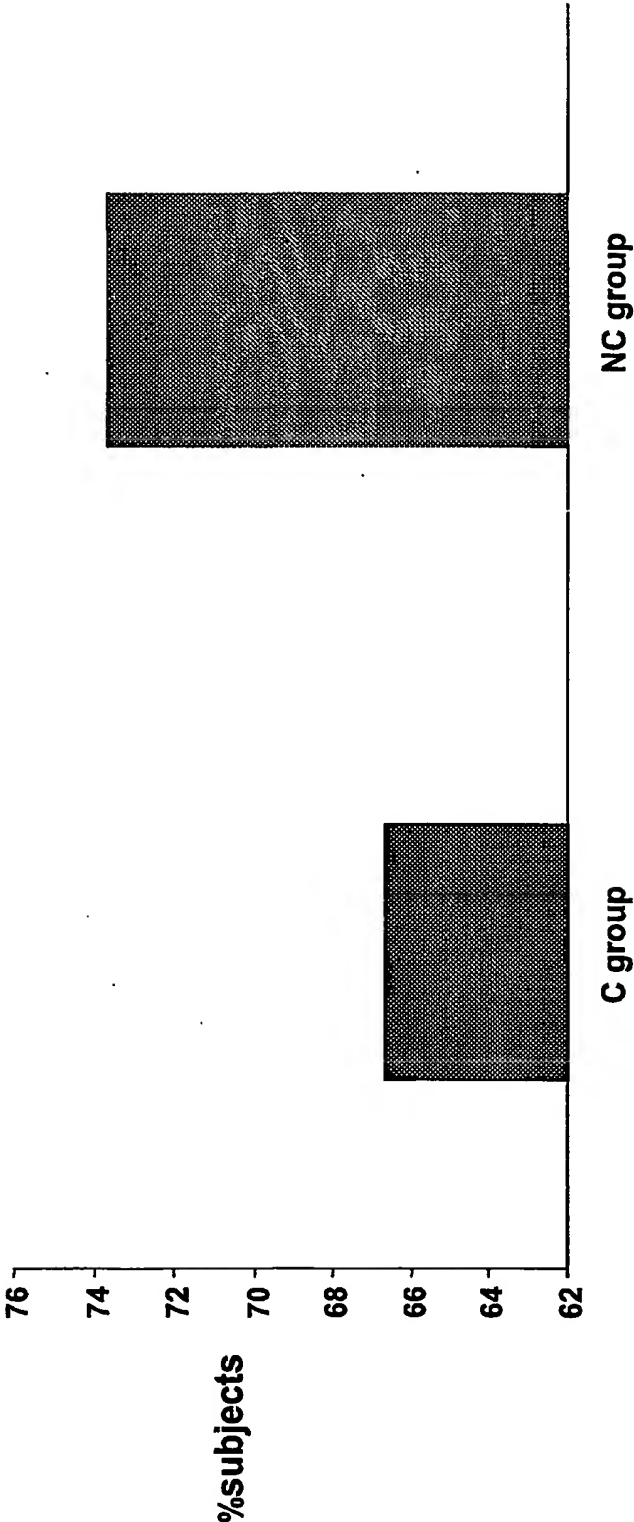
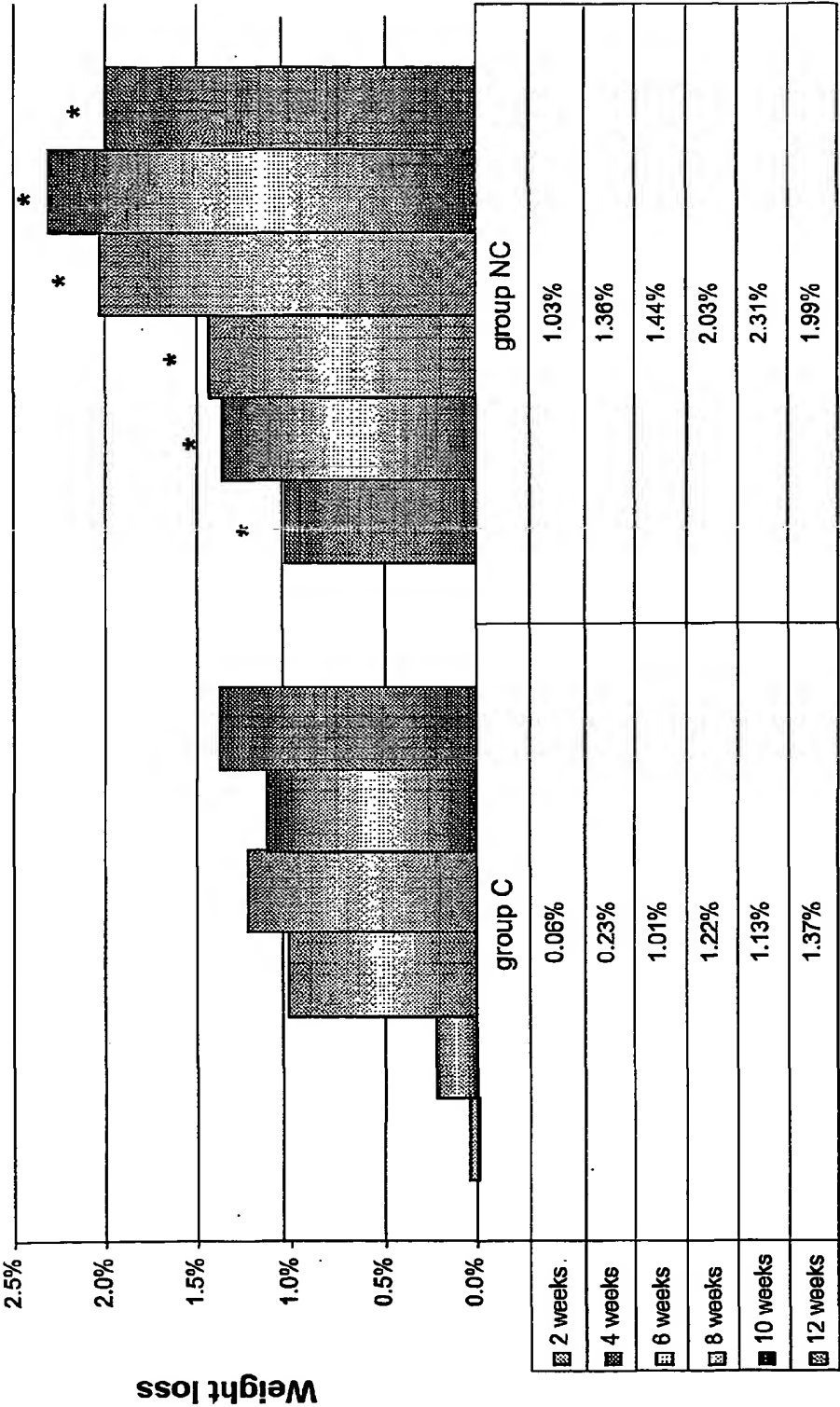


Figure 2

**Average percentage of study subjects
who experienced weight loss**



Percentage of weight loss in groups C and NC in consecutive study time intervals



* Statistically significant difference (p<0.01)

Figure 3

Percentage of weight loss in group C and NC at consecutive study time intervals

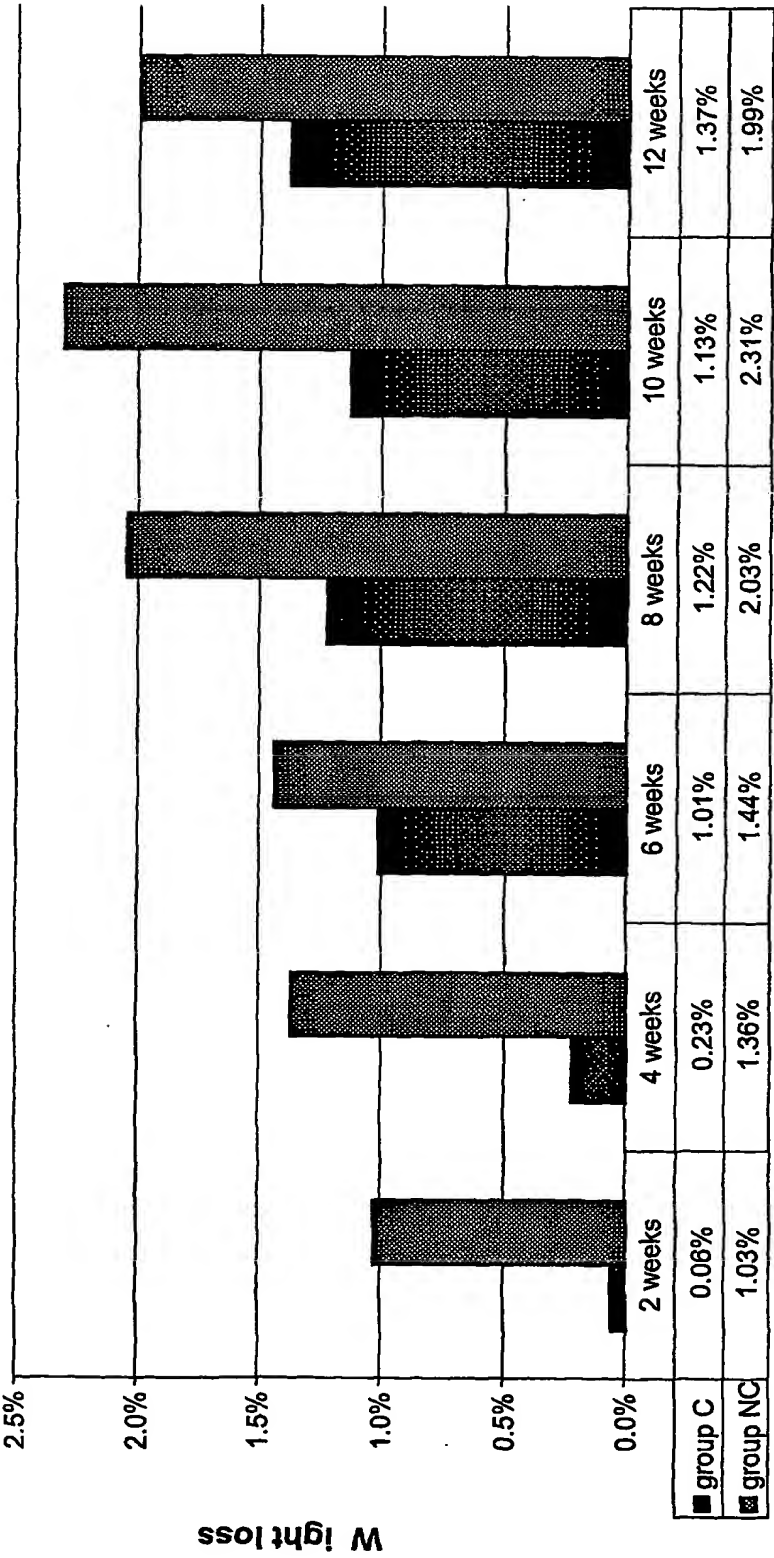
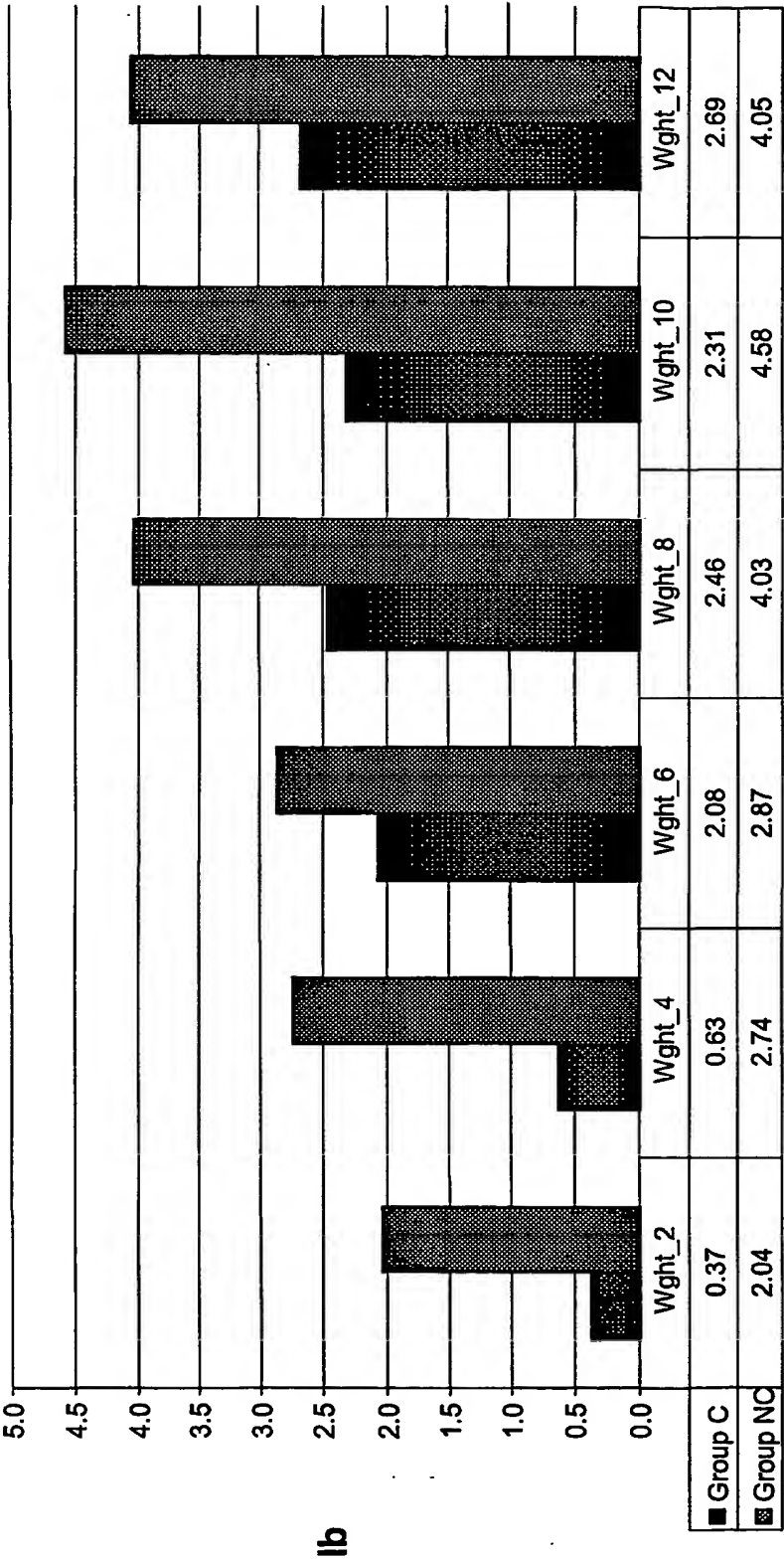


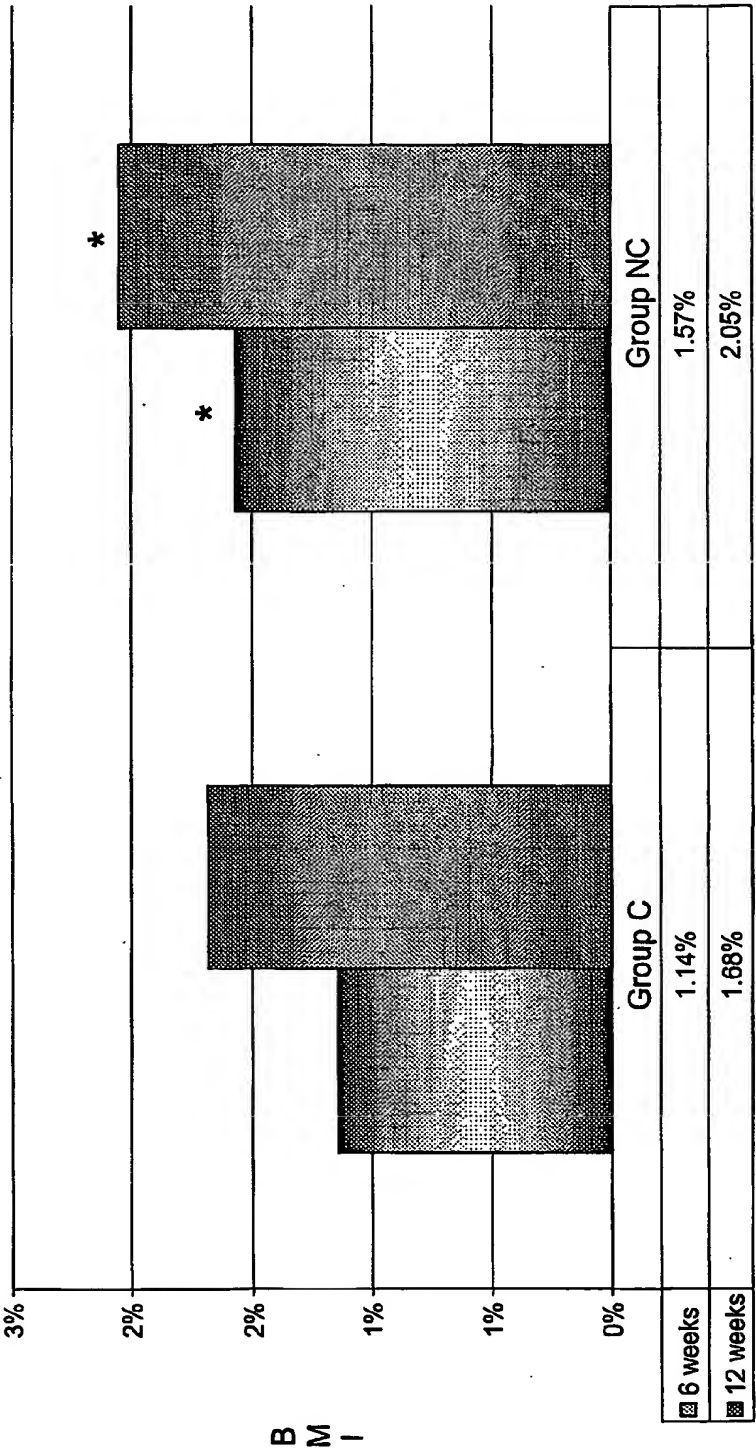
Figure 4

Figure 5

Average weight loss (lbs) in group C and NC at consecutive study intervals



**Average percentage body mass index (BMI)
change in groups C and NC at two study time
intervals**

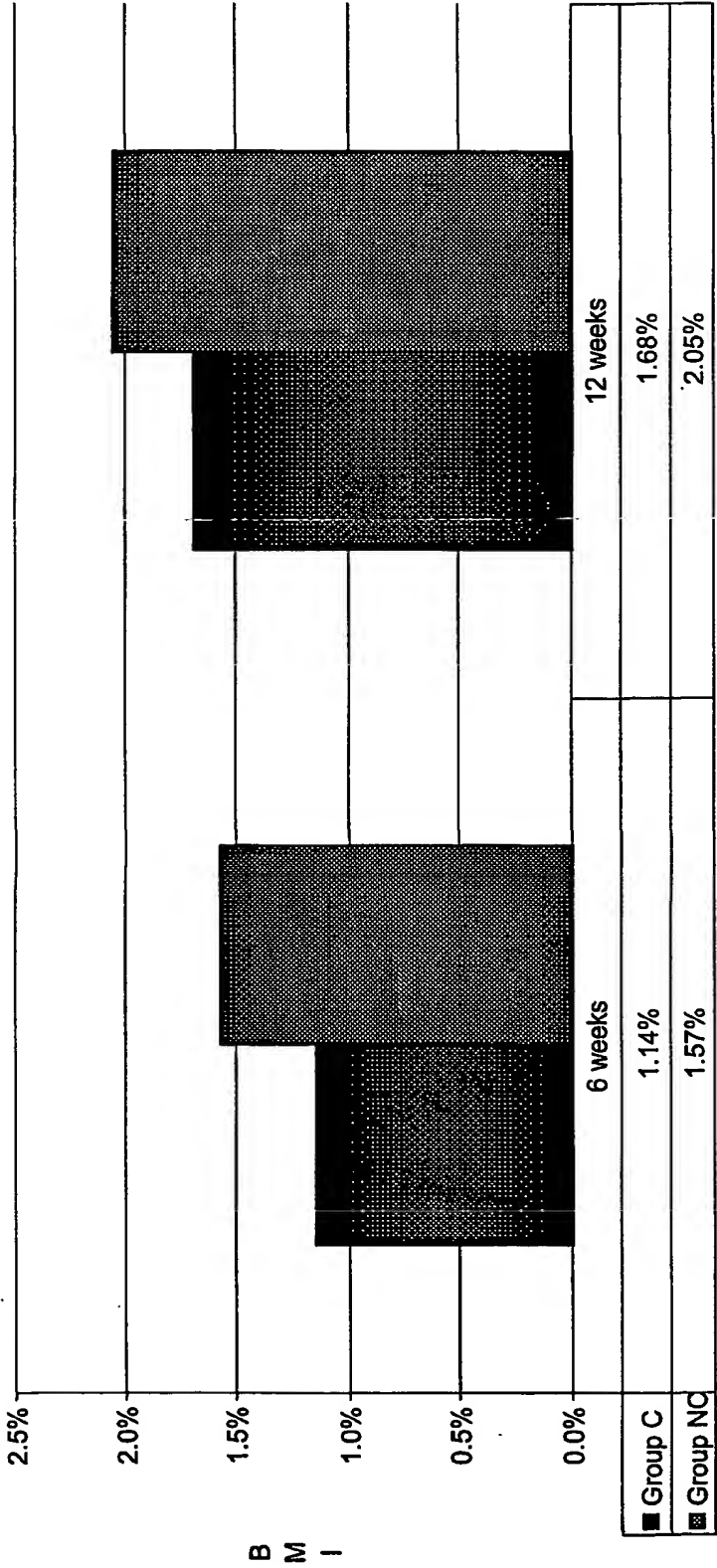


* Statistically significant difference (p<0.01)

Figure 6

Figure 7

Percentage of body mass index (BMI)
change in group C and NC
at two study time intervals



Percentage body weight change in group C subjects

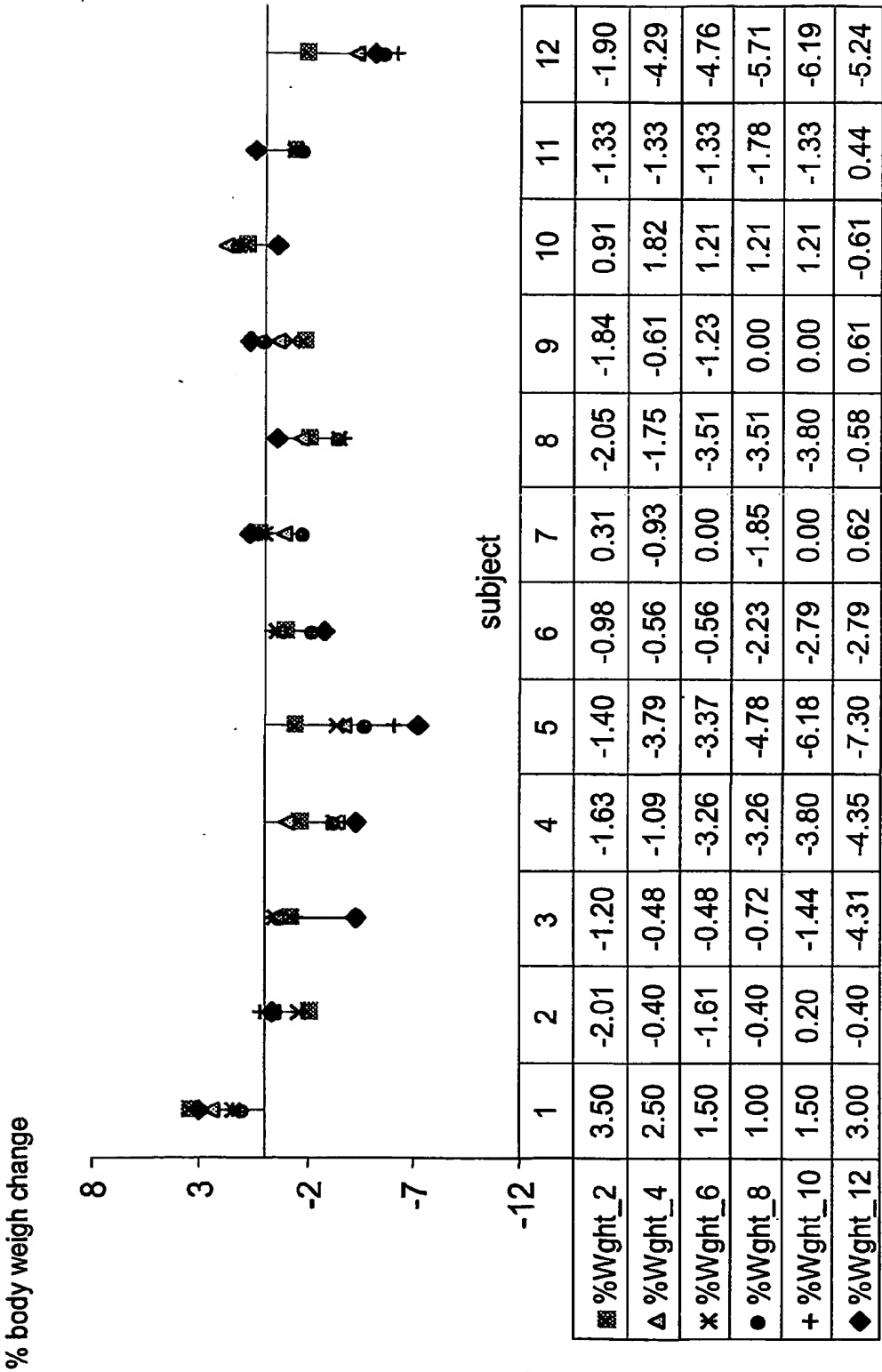


Figure 8

Percentage body weight change in NC group subjects

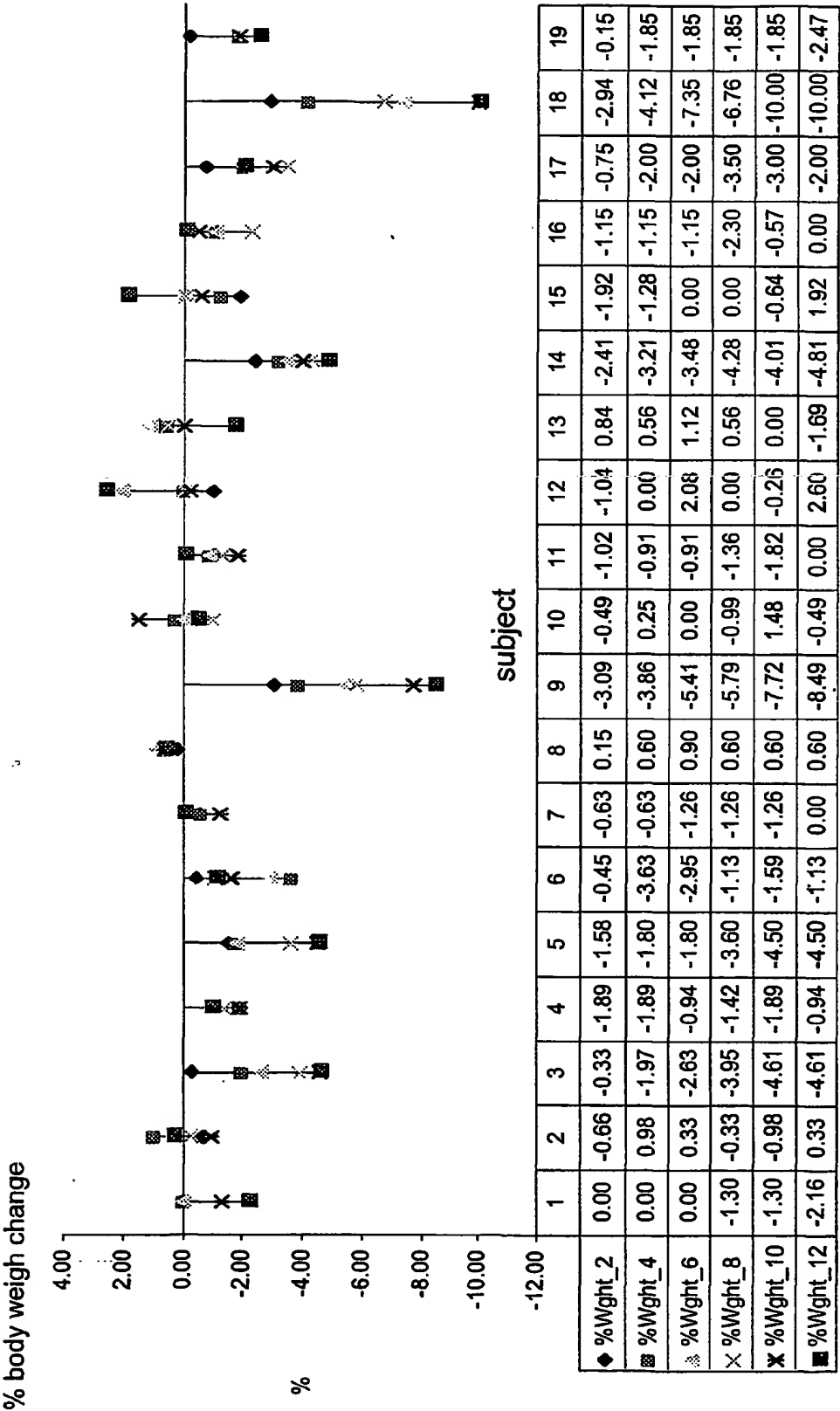


Figure 9

Percentage lean body mass (LBM) change in
group NC subjects

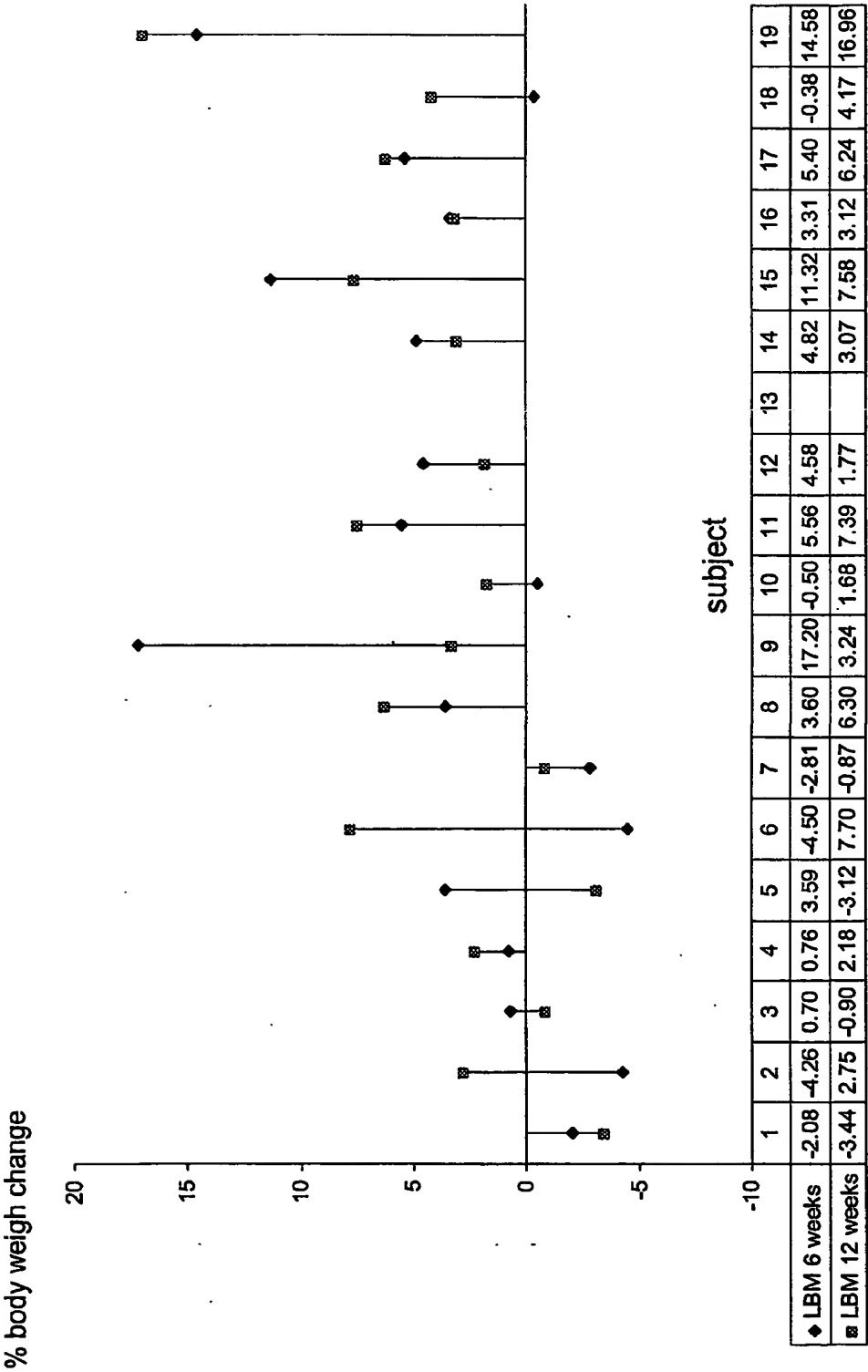


Figure 10

Figure 11

Percentage lean body mass (LBM) change in group C subjects

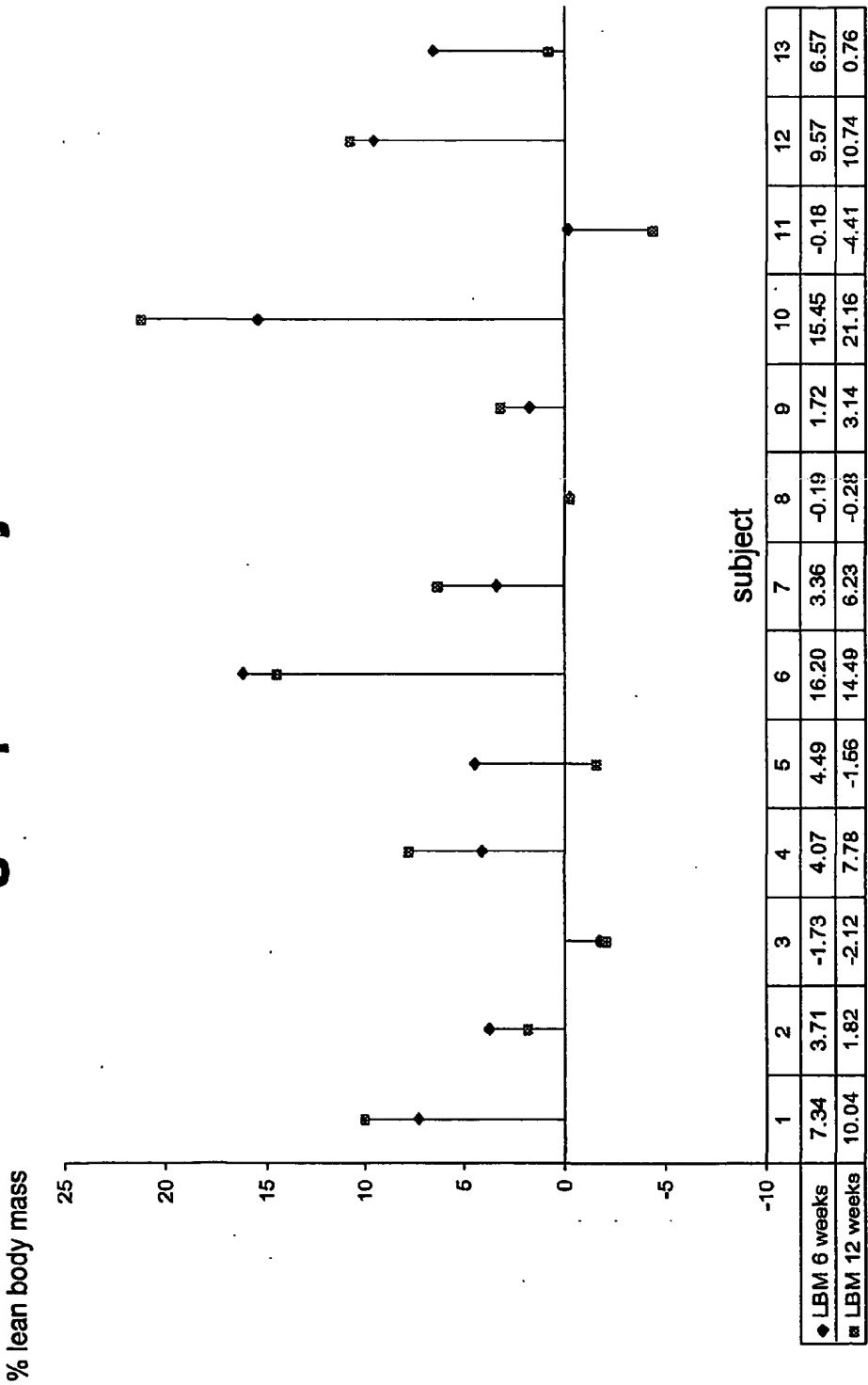


Figure 12

Percentage fat loss in group NC subjects

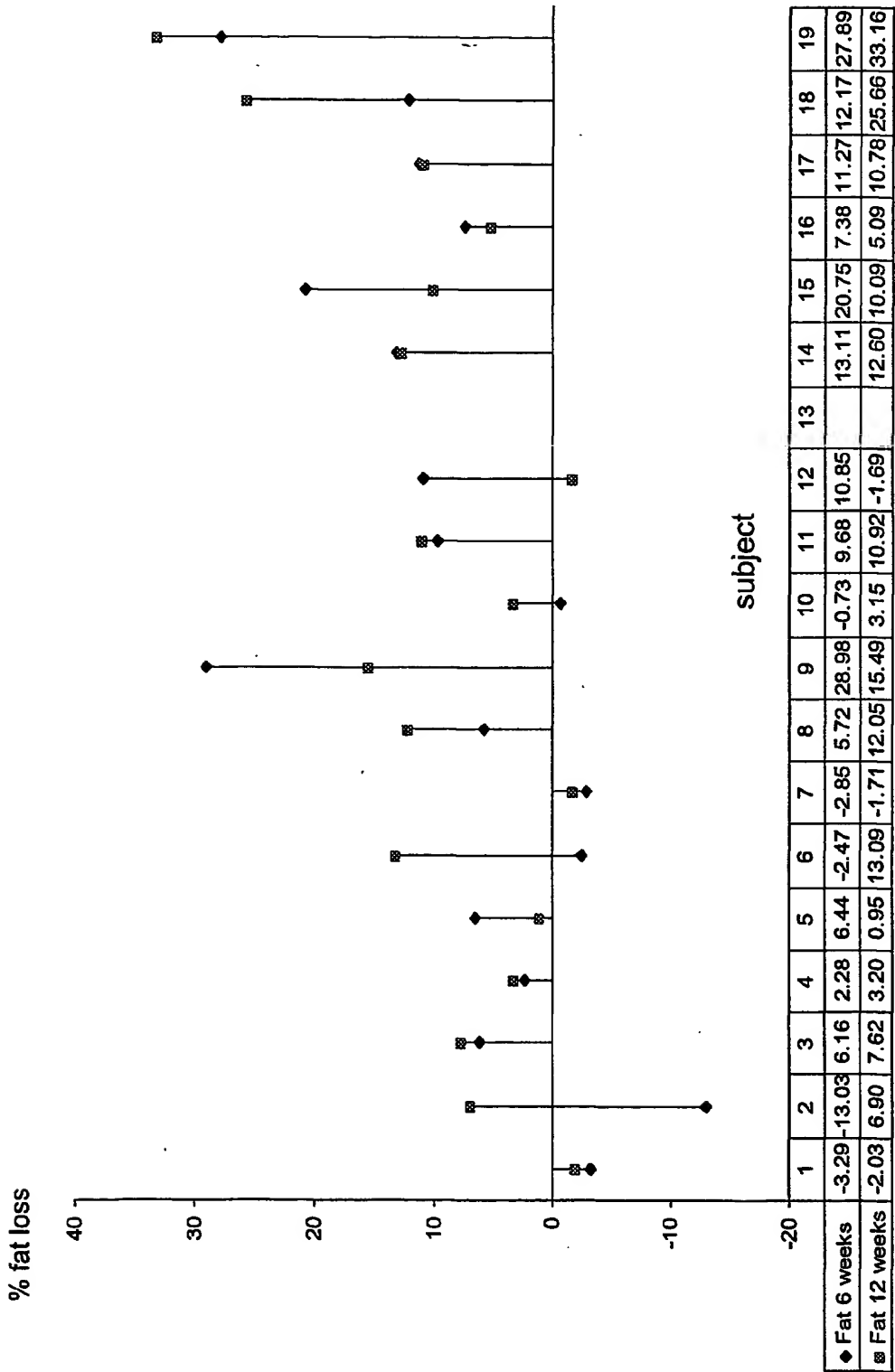


Figure 13

Percentage water change in group C subjects

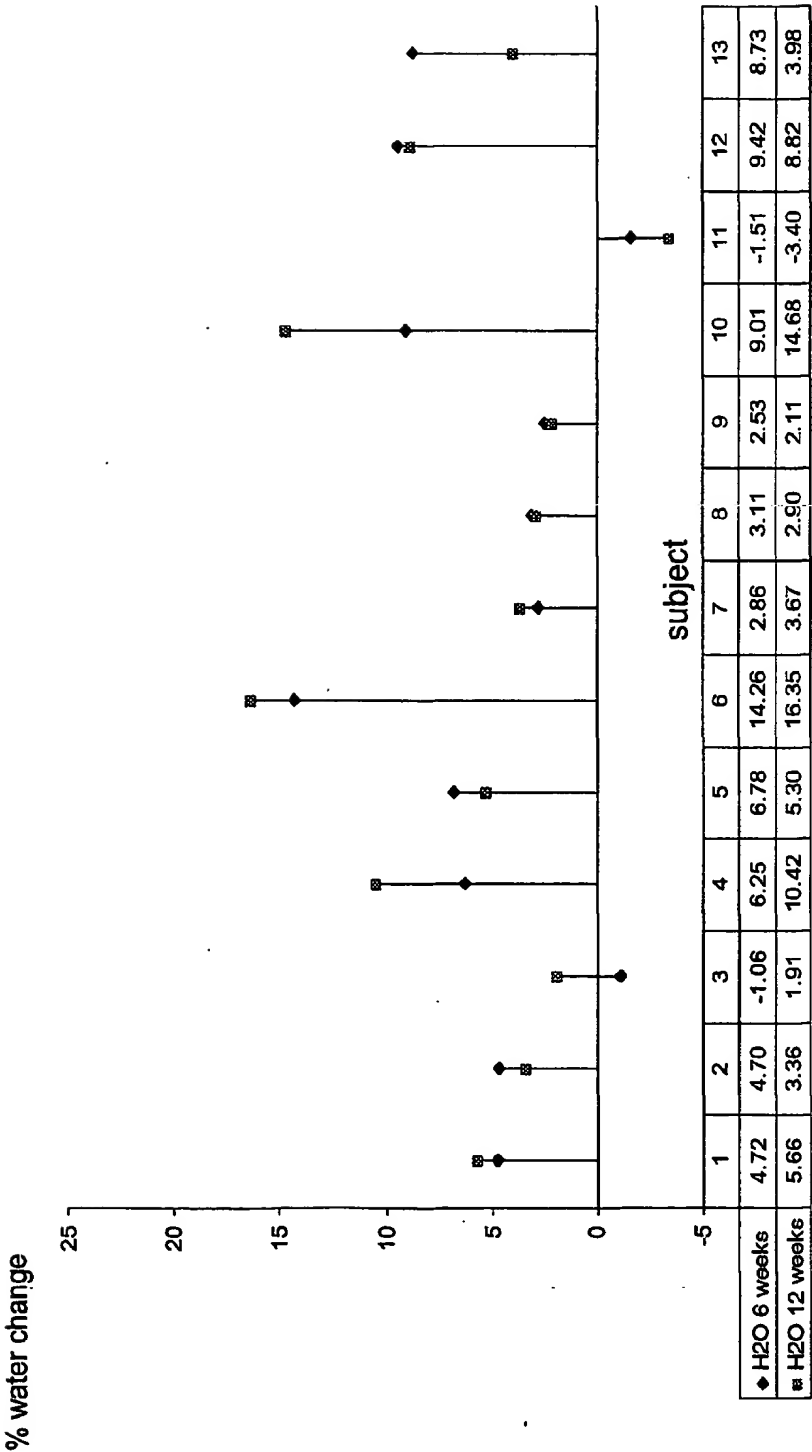


Figure 14

Percentage water change in group NC subjects

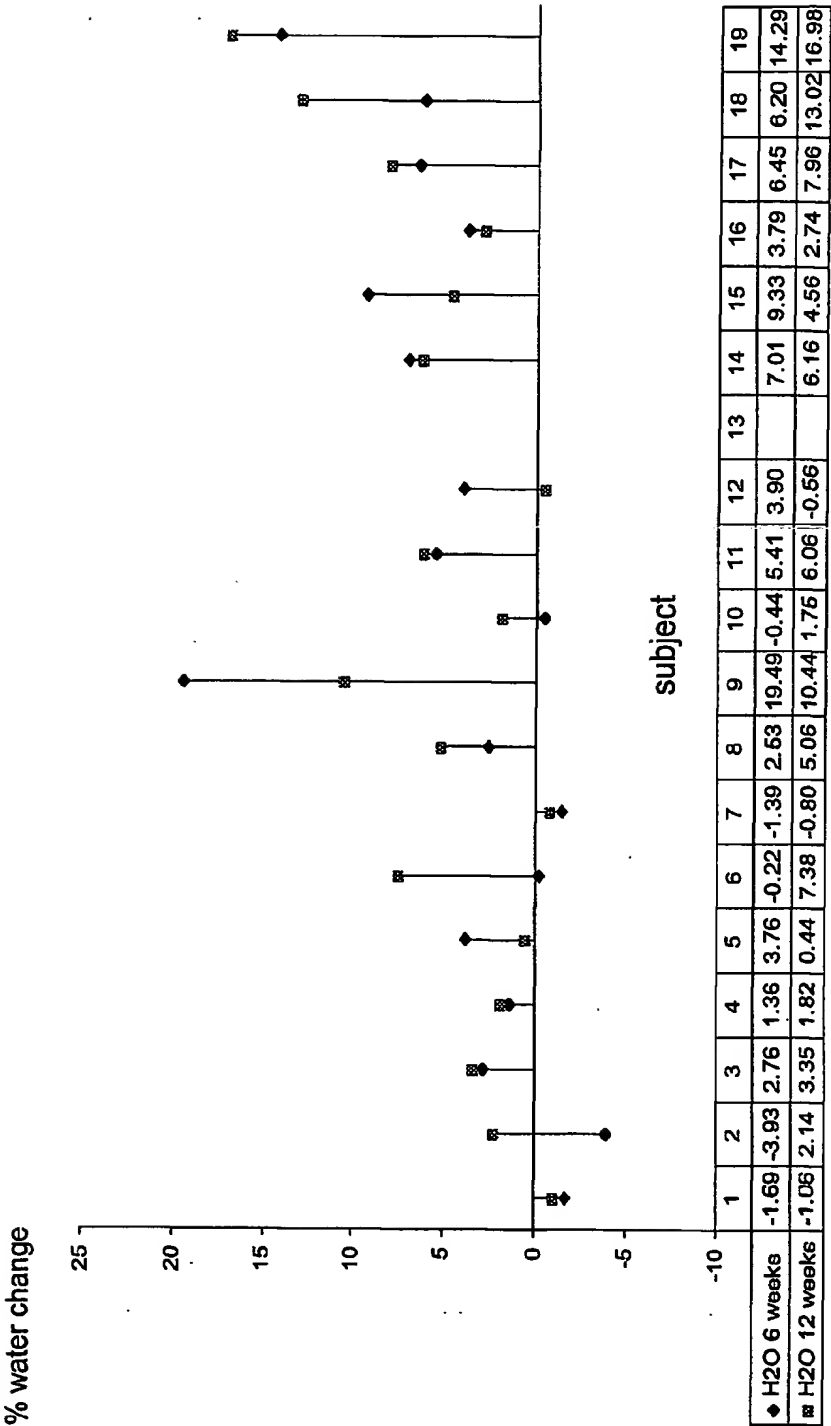
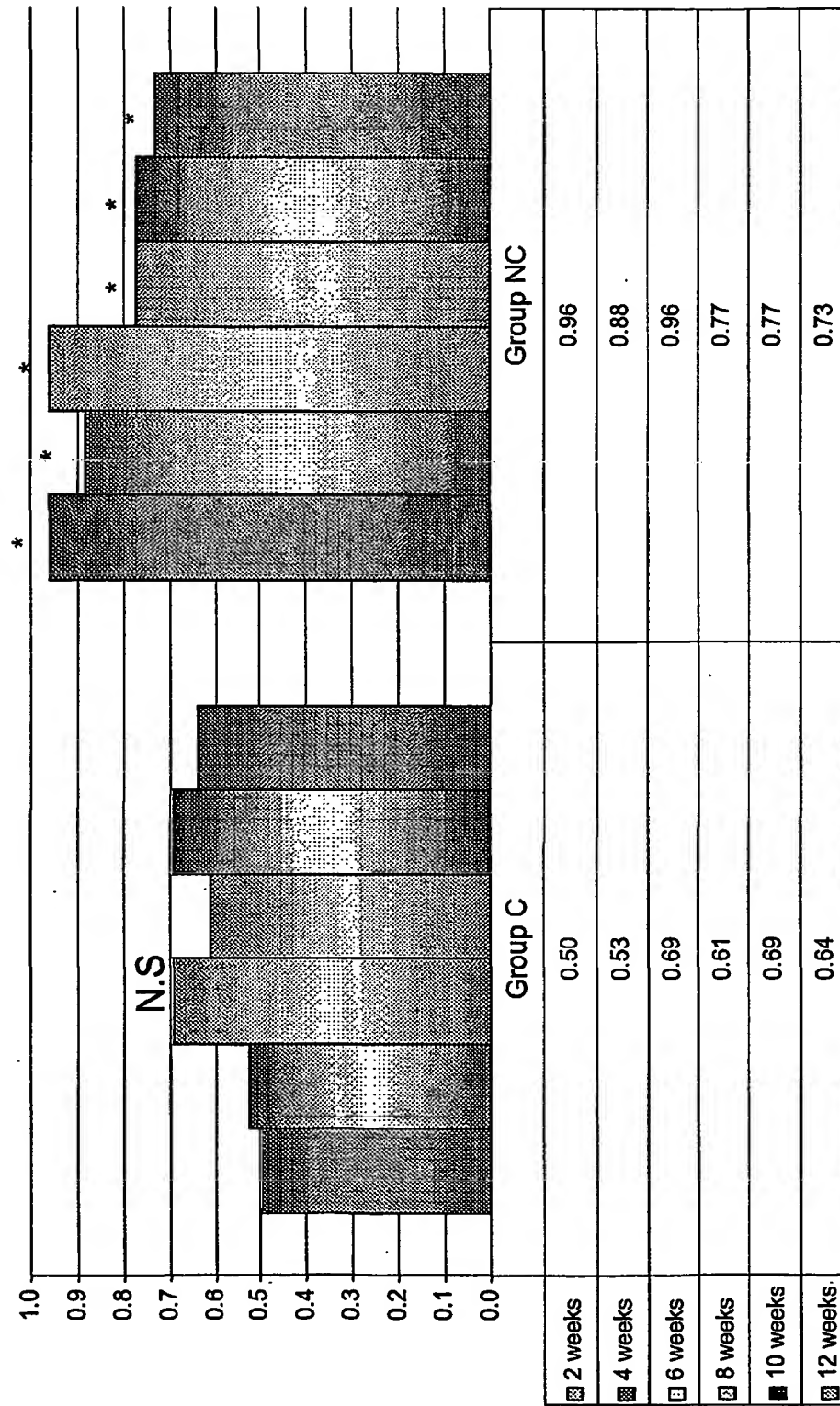


Figure 15

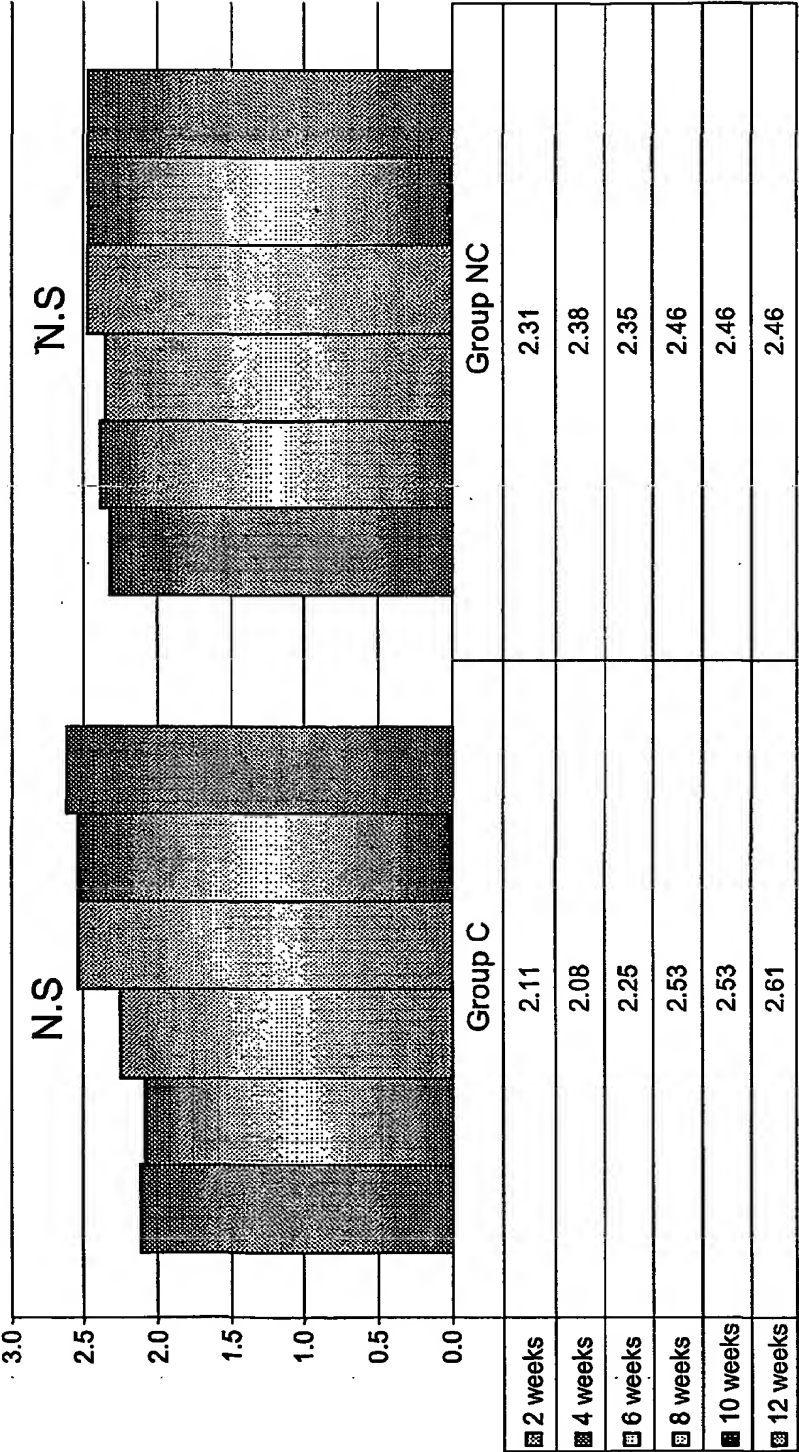
Self assessed appetite levels in group C and NC in consecutive time intervals



* Statistically significant difference ($p < 0.01$)

N.S.- not significant

Self assessed energy levels in groups C and NC in consecutive study time intervals

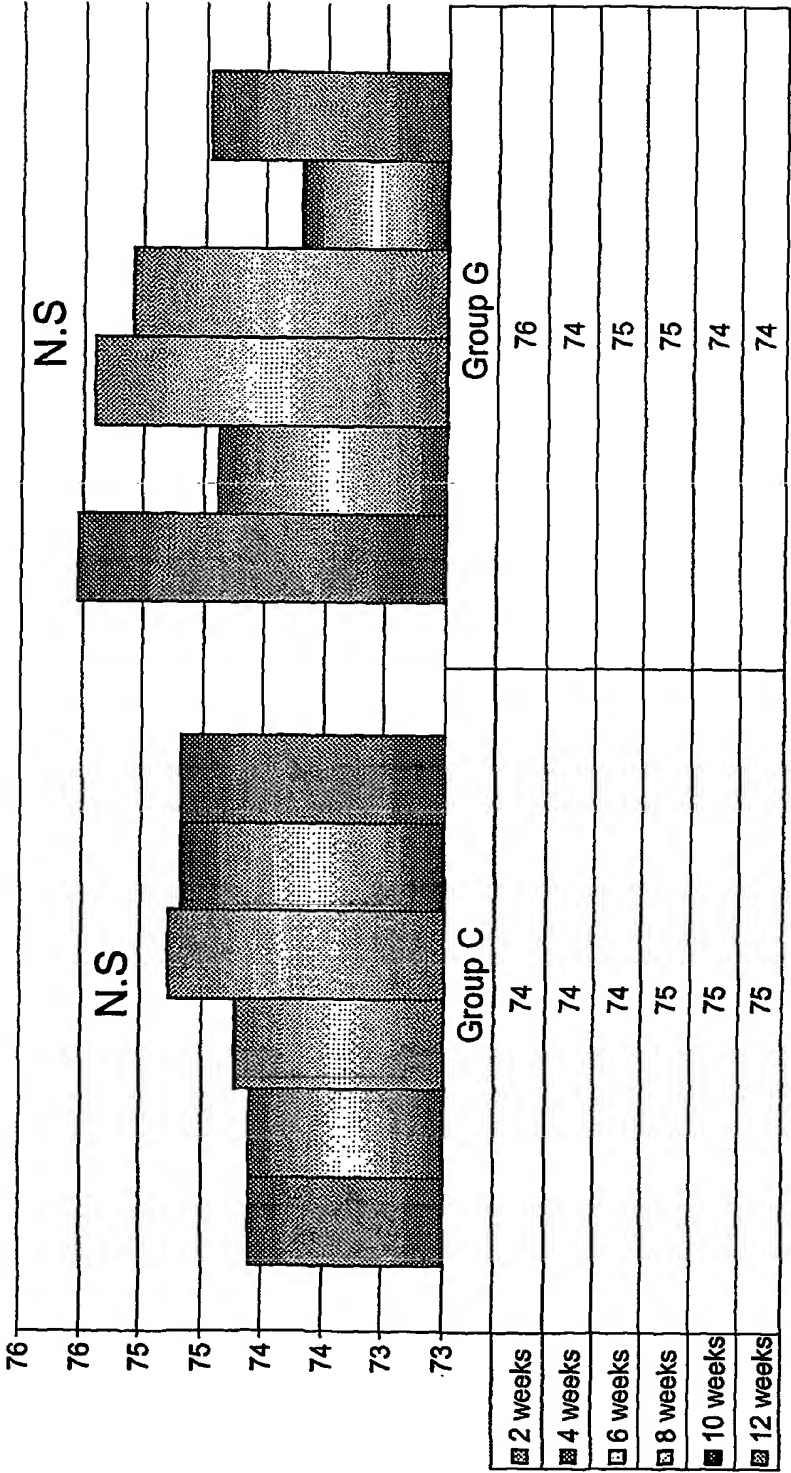


N.S.- not significant

Figure 16

Figure 17

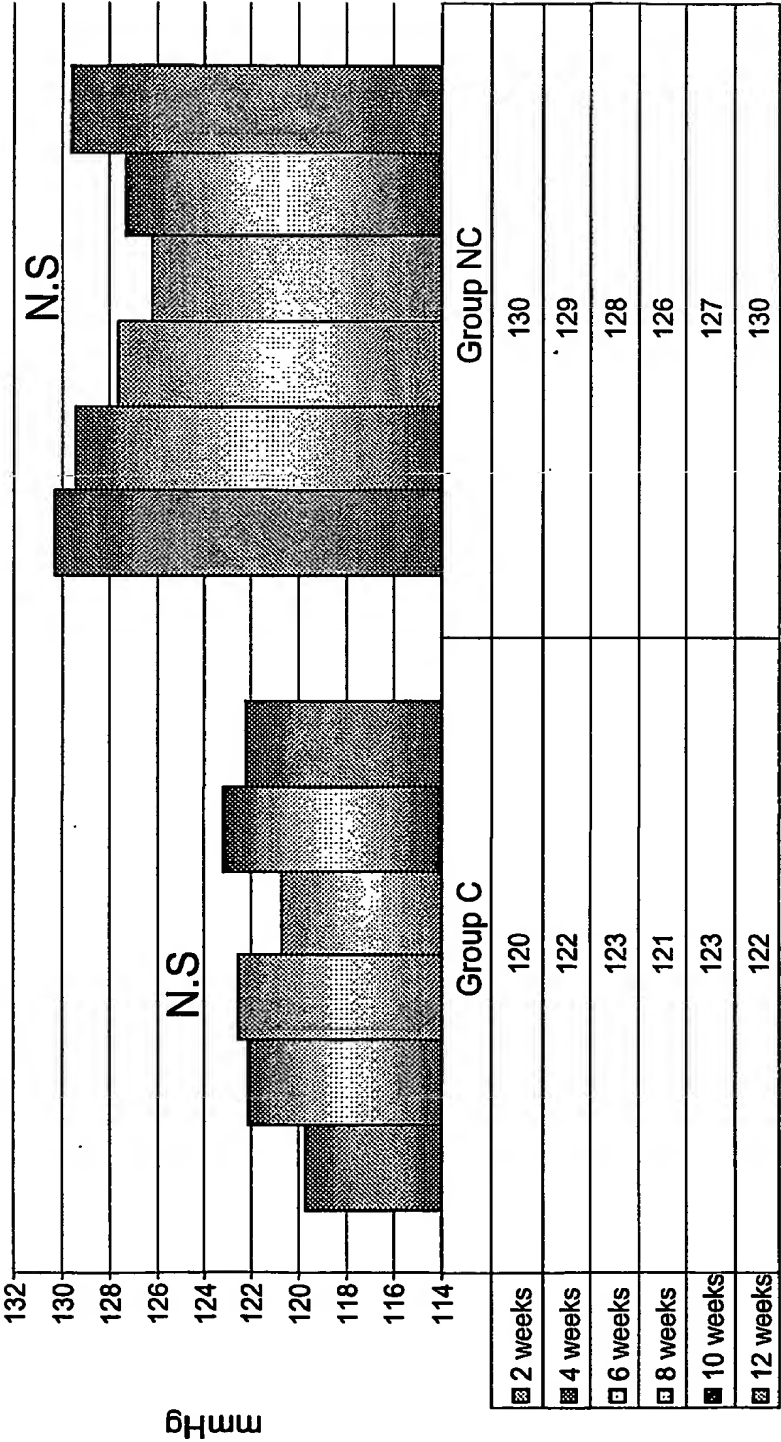
Pulse rate in groups C and NC in consecutive time intervals



N.S. - not significant

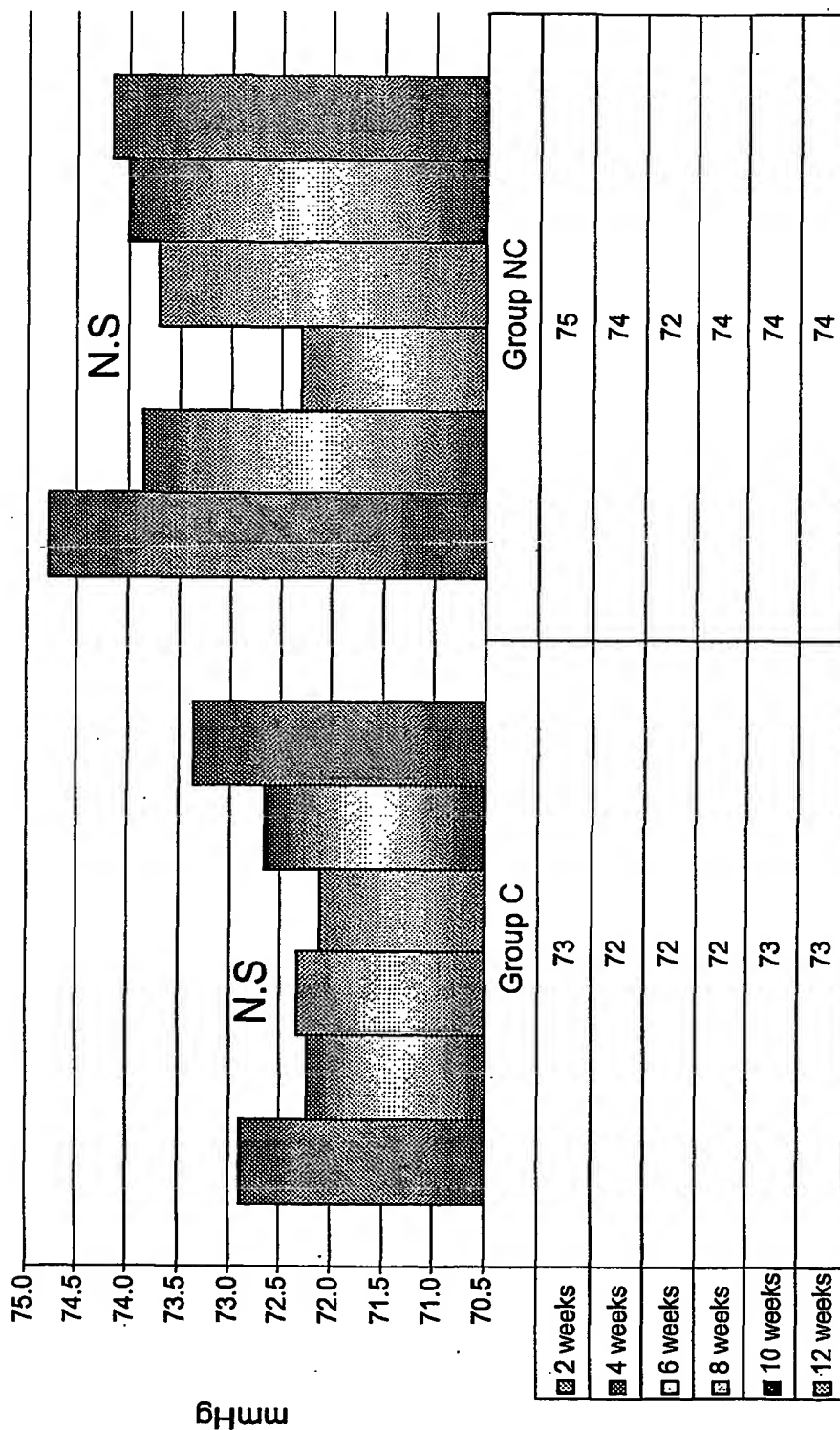
Systolic blood pressure in groups C and NC in consecutive study time intervals

Figure 18



N.S.- not significant

Diastolic blood pressure in groups C and NC in consecutive study time intervals



N.S.- not significant

Figure 19

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For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.

(54) Title: BIOAVAILABLE COMPOSITION OF NATURAL AND SYNTHETIC HCA

(57) Abstract: The invention relates to a composition comprising hydroxycitric acid (HCA) in combination with either one or both of garcinol and anthocyanin, and its use as a weight-loss therapy in animal subjects, preferably humans. The therapeutics effects for the composition observed in murine and human studies include a reduction in total body weight and body mass index, a reduction in body fat, an increase in lean body mass and content of body water, and a reduction in perceived appetite level. Another composition for use in weight-loss therapy is also described relating to forskolin in combination with either one or both of garcinol and anthocyanin. The anti-oxidant properties of garcinol are described as being enhanced in the presence of HCA and anthocyanin, and the combination of HCA, garcinol and anthocyanin is also shown to exert greater citrate lyase inhibiting properties than either compound alone. Methods of obtaining HCA, garcinol or anthocyanin, or a composition containing all three compounds, are described.

WO 02/014477 A3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/41748

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61K 9/20, 9/48, 9/68, 47/12

US CL : 424/439, 440, 451, 464

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/439, 440, 451, 464

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Database Caplus on STN, Accession Number 2000:89608, MATTES et al., Effects of (-)-Hydroxycitric Acid On Appetitive Variables, Physiology & Behavior 2000, 71, No. 1/2, CODEN:PHBHA4; ISSN: 0031-9384, pages 87-94.	1-3



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier document published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"A" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

15 MARCH 2002

Date of mailing of the international search report

30 MAY 2002

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/41748

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-3

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s) 1-3, drawn to a method for appetite suppression comprising hydroxycitric acid and garcinol.
Group II, claim(s) 4-6, drawn to a method for inhibiting cytoplasmic citrate lyase comprising HCA and anthocyanin.
Group III, claim(s) 7-9, drawn to a method for increasing fat metabolism comprising HCA and garcinol.
Group IV, claim(s) 10-11, drawn to a method of increasing antioxidant activity of garcinol.
Group V, claim(s) 12, 15, 16, and 19, drawn to a process of manufacturing hydroxycitric acid.
Group VI, claim(s) 13, 17, and 19, drawn to a process of manufacturing garcinol.

Group VII, claim(s) 14, 18, and 19, drawn to a method of manufacturing anthocyanin.
Group VIII, claim(s) 15 and 19, drawn to a method of manufacturing HCA, garcinol, and anthocyanin.
Group IX, claim 20, drawn to a method of appetite suppression comprising administering forskolin and gascinol.
Group X, claim(s) 21, drawn to a method of appetite suppression comprising administering forskolin and anthocyanin.
Group XI, claim(s) 22, drawn to a method of appetite suppression comprising administering forskolin, anthocyanin, and garcinol.
Group XII, claim(s) 23, drawn to a method of suppressing cytoplasmic forskolin and garcinol.
Group XIII, claim(s) 24, drawn to a method of suppressing cytoplasmic citrate lyase but=y administering forskolin and anthocyanin.

Group XIV, claim(s) 25, drawn to a method of suppressing cytoplasmic citrate lyase by administering forskolin, garcinol, and anthocyanin.
Group XV, claim(s) 26, drawn to a method of increasing fat metabolism by administering forskolin and garcinol.
Group XVI, claim(s) 27, drawn to a method of increasing fat metabolism by administering forskolin and anthocyanin.
Group XVII, claim 28, drawn to a method of increasing fat metabolism by administering forskolin, garcinol, and anthocyanin.

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s) 1-3, drawn to a method for appetite suppression comprising hydroxycitric acid and garcinol.
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Group V, claim(s) 12, 15, 16, and 19, drawn to a process of manufacturing hydroxycitric acid.
Group VI, claim(s) 13, 17, and 19, drawn to a process of manufacturing garcinol.

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Group XVII, claim 28, drawn to a method of increasing fat metabolism by administering forskolin, garcinol, and anthocyanin.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/41748

The inventions listed as Groups do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: Groups I-XVII differ not only in the claimed methods, but also in the different biologically active agents used in the same claimed methods. As such, Groups I-XVII lack the same special technical features.